

20 December 2013 | \$10

Science

Breakthrough of the Year

Cancer Immunotherapy

T cells on the attack



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Breakthrough of the Year

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>> Breakthrough Online

Check out all the science highlights from 2013, including videos on the breakthrough, our genomes-of-the-year slideshow, and a special year-end podcast at www.sciencemag.org.



COVER

Antibodies (pink) zoom toward a T cell (gray, with CTLA-4 receptor proteins shown in light blue), giving the T cell a push to attack tumor cells. In 2013, new therapies targeting the immune system to treat cancer surged ahead, with promising but still preliminary results in people with many forms of the disease. See the Breakthrough of the Year special section beginning on page 1431 and at <http://scim.ag/Breakthrough13>.

Image: Valerie Altounian/Science

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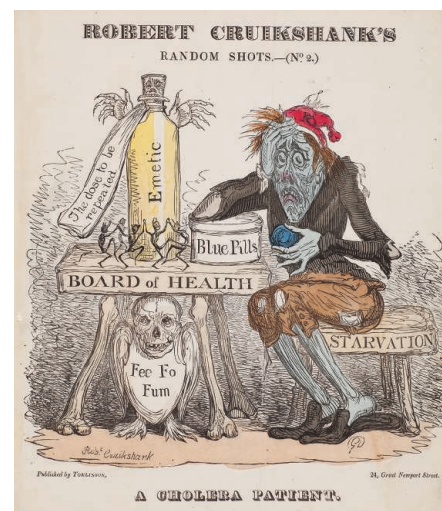
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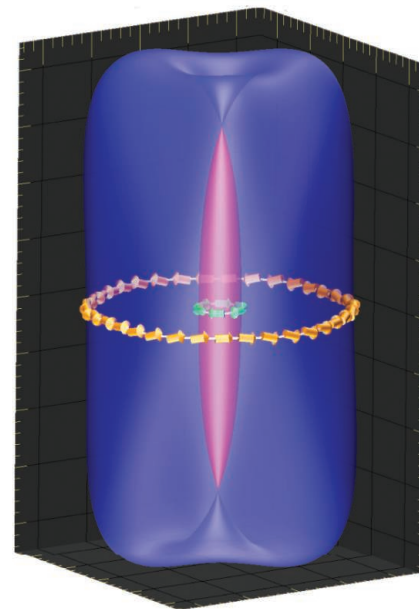
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<< Shaping Plant Evolution

Amborella trichopoda is understood to be the most basal extant flowering plant and its genome is anticipated to provide insights into the evolution of plant life on Earth (see the Perspective by Adams). To validate and assemble the sequence, Chamala *et al.* (p. 1516) combined fluorescent in situ hybridization (FISH), genomic mapping, and next-generation sequencing. The Amborella Genome Project (p. 1467) was able to infer that a whole-genome duplication event preceded the evolution of this ancestral angiosperm, and Rice *et al.* (p. 1468) found that numerous genes in the mitochondrion were acquired by horizontal gene transfer from other plants, including almost four entire mitochondrial genomes from mosses and algae.

Weighing Up Exoplanets

The mass of a planet is important to know, but it is difficult to determine for an exoplanet. If a transmission spectrum of an exoplanet is available, de Wit and Seager (p. 1473) show that it is possible to determine its mass based on the properties of its atmosphere. The method is suited for low-density planets orbiting bright or large stars, and it is complementary to other mass-retrieval methods.

Knowing the Enemy

Infection of host cells by HIV-1 is mediated by an envelope glycoprotein (Env) trimeric spike on the surface of the virus. Proteins comprising the Env trimer must be cleaved for infectivity, and thus viral fusion involves three Env conformations. The flexibility of the Env trimer has made it a challenge to determine a high-resolution structure, although such a structure is key both for understanding trimer function and for guiding vaccine design. Lyumkis *et al.* (p. 1484) and Julien *et al.* (p. 1477) studied soluble cleaved trimers stabilized by specific mutations but that have kept a near-native antigenicity profile. Lyumkis *et al.* present a high-resolution structure of the trimer in complex with a broadly neutralizing antibody, and Julien *et al.* present a crystal structure of the trimer in complex with another broadly neutralizing antibody.

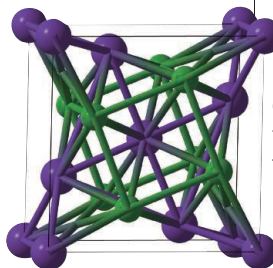
Relaxing in a Water Jet

High-energy irradiation of liquid water and its solutes can transiently liberate electrons, which act as potent chemical reductants, but they are challenging to characterize precisely. Seeking to bridge the gap between liquid and gas, Elkins *et al.* (p. 1496) report results from photoelectron

spectroscopy of hydrated electron dynamics in a liquid jet. The results reveal a very rapid transition from the electronic excited state to the ground state, prior to full relaxation of the solvent shell.

Salt to Squeeze

Simple table salt, NaCl, is the only known stable phase of Na and Cl at ambient conditions. Previous attempts to understand its structure and chemical properties under pressure and at high temperatures revealed phase and bonding transitions, while keeping the balance of one Na to one Cl. Using crystal structure prediction algorithms, Zhang *et al.* (p. 1502; see the Perspective by Ibáñez Insa) show that other compounds—including Na₃Cl, Na₂Cl, Na₃Cl₂, NaCl₃, and NaCl₄ are as stable as NaCl across a range of pressures.



Power in Numbers

Avian brood parasites target particular bird species to raise their offspring, sometimes at great cost to the foster family. Feeney *et al.* (p. 1506; see the Perspective by Spottiswoode) analyzed the global distribution of brood parasitism and found a correlation with the occurrence of cooperative breeders across multiple taxa. For example, Australian fairy wrens breed both singly and in cooperative groups, but the group breeders are better able to resist parasites than lone pairs, indicating that the prevalence of cooperative breeding may be a response to brood parasites.

G Structures

G protein-coupled receptors (GPCRs) are eukaryotic membrane proteins that have a central role in cellular communication and have become key drug targets. To overcome the difficulties of growing GPCRs crystals, Liu *et al.* (p. 1521) used an x-ray free-electron laser to determine a high-resolution structure of the serotonin receptor from microcrystals.

An Enzyme Drill

Cellulase enzymes degrade the cell walls of plants by breaking down cellulose into its constituent sugar fragments and thus have attracted interest for biofuels production. Using transmission electron microscopy Brunecky *et al.* (p. 1513; see the Perspective by Berlin) discovered that an especially active cellulase, CelA, from *Caldicellulosiruptor bescii* bacteria does not move along the surface of the substrate, but drills into the cellulose to form cavities.

Ubiquitin Fertility Insurance

The female mammal's reproductive lifespan is determined by a pool of ovarian primordial follicles that are generated early in life. Yu *et al.* (p. 1518) found that in mice, the ubiquitin E3 ligase complex CRL4 is essential for oocyte survival within primordial follicles and for development after fertilization. CRL4 binds to and activates an adaptor protein that mediates ubiquitination, but if any component is deleted, the genes required for oocyte maintenance and early embryo development are silenced and the female mice become infertile.

Additional summaries

Spin Berry's Phase

When a quantum mechanical system performs an adiabatic cyclic path in the space of the parameters that affect its state (such as, for example, the magnetic field) its wave function may acquire an additional phase rather than go back to its original value. This quantity, called the Berry's phase, is associated with the topological properties of the parameter space and has been observed in materials such as graphene and bismuth. **Murakawa *et al.*** (p. 1490) observe a Berry's phase equal to π in the material BiTeI in which the phenomenon is predicted to be a consequence of a very strong coupling of spin and orbital degrees of freedom realized through the so-called Rashba effect.

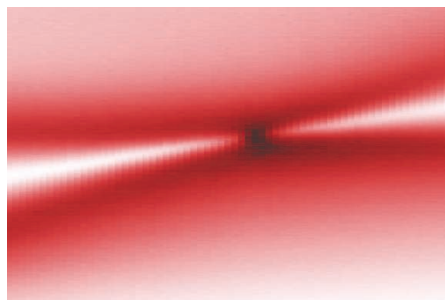
Access via Vibration

Molecular beam studies over the past decade have elucidated many subtle quantum mechanical factors governing the influence of vibrational excitation on the outcome of elementary chemical reactions. However, these studies have generally had to focus on reagents that can be easily made to vibrate by direct absorption in the infrared (IR). **Wang *et al.*** (p. 1499) show that a variation on stimulated Raman pumping can efficiently excite the IR-inactive stretch vibration in the diatomic molecule, hydrogen deuteride (HD). As a result, they can probe the influence of that vibration on the outcome of the HD + F reaction. Through a combined spectroscopic and

theoretical investigation, they uncover Feshbach resonances along the reaction coordinate that are only accessible through vibrational pre-excitation.

Artificial Complexity

Quantum optics probes the interactions between light and matter. Building up from a simple, single-atom system, the exchange of virtual photons between systems of several (or many)



atoms is expected to give rise to many exotic effects. Because controlling the separation of the atoms on the atomic scale is experimentally challenging, artificial atom systems may provide a more tractable route for systematic study, as described by **van Loo *et al.*** (p. 1494, published online 14 November). Using a system of two separate superconducting qubits in a microwave transmission line, they show how the interaction between the two qubits can be controlled and mediated by electromagnetic modes. The

results illustrate a feasible route to probing the complexity of many-body effects that may otherwise be difficult to realize.

Not All Mice Are Equal

Different laboratories often use different strains of inbred animals, but one cannot make behavioral comparisons and assume that their reaction to interventions will necessarily be similar. **Kumar *et al.*** (p. 1508) have detected differences in cocaine response between the widely used C57BL/6N and C57BL/6J mouse strains and used quantitative trait locus analysis to identify a mutation in an inducible gene, *Cyfp*, that interacts with the Fragile X protein (FMRP) to regulate sensitivity and sensitization to cocaine through regulation of neuronal connectivity.

More from mTOR

Leigh syndrome is a rare, untreatable, inherited neurodegenerative disease in children that is caused by functional disruption of mitochondria, the cell's energy-producing organelles. **Johnson *et al.*** (p. 1524, published online 14 November; see Perspective by **Vafai and Mootha**) show that rapamycin, a drug used clinically as an immunosuppressant and for treatment of certain cancers, delayed the onset and progression of neurological symptoms in a mouse model of Leigh syndrome and significantly extended survival of the animals. Rapamycin inhibits the so-called "mTOR" signaling pathway, which is currently under intense study because it plays a contributory role in many common diseases.



Marcia McNutt is Editor-in-Chief of *Science*.

Cancer Immunotherapy

THE WAR ON CANCER BEGAN A LITTLE MORE THAN 40 YEARS AGO AS A NATIONAL RESEARCH PROGRAM to radically improve the survival of patients with cancer, a leading cause of death in the United States and worldwide. The main weapons deployed have been surgery, radiation, and chemotherapy, treatments that often carry risks and/or cause adverse side effects. Although some forms of cancer yield to these therapies, not all do, and thus mortality remains high.

To that list we now add a fourth weapon, cancer immunotherapy. Constructed over decades, it has begun to demonstrate such promising results in cancer patients that we have selected it as the Breakthrough of the Year for 2013. The choice of a topic that is clinical in nature is something of a departure for *Science*. But we believe that 2013 marks a significant moment in cancer history, and today's achievements merit recognition and celebration, even if uncertainties remain. With more people now living well beyond age 65, the incidence of cancer is projected to rise steeply in the coming years. Thus, the population who might benefit from immunotherapy is potentially quite large.

Cancer immunotherapy aims to harness the body's own immune system to fight cancer. Today's successes are rooted in fundamental research beginning in the late 1980s in the labs of James Allison and others to decipher protein receptors that put the brakes on T cells (see the News story on p. 1432). Cancer researchers hypothesized that if these receptors could be blocked, the immune system might attack cancerous cells. At least in principle, such immune-based therapies would offer two advantages over other cancer drugs: These therapies could be applied to a diverse range of tumor types, and patients would not be expected to develop resistance to them.

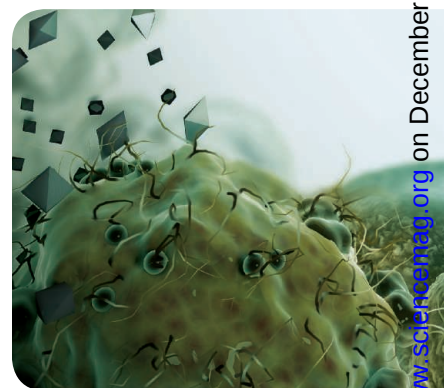
Research ultimately led to the development of several antibody therapies, one of which is now on the market. Meanwhile, on another front, researchers are genetically engineering T cells to target tumor cells. Although dozens of clinical trials are still under way, the results are encouraging: Some patients with end-stage metastatic disease that had not responded to other aggressive therapies are surviving for much longer than doctors would predict. A paper published in July* reported that among 52 people with advanced melanoma, tumors shrank in 21 of those receiving a combination of two antibodies. More recent findings presented at meetings this fall suggest that immunotherapy's promise is holding up, although there are many uncertainties and side effects from some of the treatments.

Certainly we have a long way to go. Some patients who initially do well later see their cancer progress and die from it. Many questions remain as to why others do not respond to immunotherapy at all. In the long run, there is always the risk that the strategy will prove disappointing for any number of reasons. The responses may not persist over the long term, and unexpected side effects may become evident as a larger number of patients receive these new treatments. Laboratory scientists are already hard at work designing new ways to make these therapies safer and more effective, as illustrated in a paper published in *Science Translational Medicine* last week.†

As Pearl Harbor was reeling from the Japanese attack of 7 December 1941, my father dropped out of his freshman year at Harvard, waived exemption for a heart murmur, and enlisted in the U.S. infantry. With free cigarettes in his rations from the government, thus began his long relationship with tobacco that ended just a few years before his death from lung cancer in 2001. Breakthroughs in cancer immunotherapy may have arrived too late for my father, but there are many cancer patients around the world whose lives could potentially be extended as we learn more about this promising new approach.

— Marcia McNutt

10.1126/science.1249481



*J. D. Wolchok et al., *N. Engl. J. Med.* **369**, 122 (2013). †V. D. Fedorov, M. Themeli, M. Sadelain, *Sci. Transl. Med.* **5**, 215ra172 (2013).

ECOLOGY

Sharks Love Their Country

The return of individuals to the place of their birth to reproduce is called philopatry, and it occurs in many vertebrate species. Understanding the level, and details, of philopatry within a given species is important for conservation planning, particularly when it involves large, imperiled, and difficult-to-handle marine vertebrates, such as sharks. Feldheim *et al.* have undertaken an at-times arduous, 19-year survey of the coastal lemon shark, *Negaprion brevirostris*, around Bimini in the Bahamas. Previous genetic data hinted that this late-maturing species shows philopatry, and this study gathered direct evidence: Six recaptured mature female sharks were all, without exception, faithful to one nursery site or the other over multiple reproductive events. Such strong local fidelity would be expected to result in some degree of population isolation at very local scales, indicating a requirement for local conservation measures to match this faithfulness. — CA

Mol. Ecol. 10.1111/mec.12583 (2013).



CELL BIOLOGY

Centriole Central

The centriole is an evolutionarily conserved organelle involved in microtubule organization. Pairs of centrioles form the centrosome, which is the major microtubule-organizing center in interphase and the mitotic cells of higher animals. Centriole number is subjected to tight regulation, and aberrant centriole numbers cause genome instability and cell proliferation defects, leading to tumorigenesis and other diseases. The centriole also forms the basal body of the cilium, a microtubule-based tail-like membrane protrusion. Epithelial cells, such as those seen lining the trachea, contain many cilia on their apical surface. How are the hundreds of centrioles required for multiciliogenesis created? Zhao *et al.* examined multicilia formation in mouse tissues and cell lines using super-resolution three-dimensional structured illumination microscopy. Multiple centrioles were produced in ring-shaped deuterosome structures. Two related genes, *Cep63* and *Deup1*, were important for the generation

of centrioles, with *Deup67* being essential for assembling the deuterosome structures required to create multiple centrioles de novo. *Cep63*, on the other hand, was more important for mother-centriole-based centriole duplication. — SMH

Nat. Cell Biol. 15, 1434 (2013).

NEUROSCIENCE

Don't Sleep on It

Sleep deprivation has long been established as a helpful tool for the treatment of patients suffering from depression.



However, how and why it works are still unknown. Functional magnetic resonance imaging (fMRI) studies have indicated that large-scale brain network connectivity, especially in the so-called default mode network, seems to be

changed in depression. Bosch *et al.* investigated whether sleep deprivation could influence this brain connectivity. They discovered that sleep deprivation decreased functional connectivity between a brain area called the posterior cingulate cortex and the bilateral anterior cingulate cortex. In contrast, connectivity between the dorsal nexus, a region that plays a crucial role in the pathophysiology of depression, and two areas within the right dorsolateral prefrontal cortex was increased. These sleep deprivation-induced changes in resting-state connectivity indicate a shift in dominance from a more affective to a more cognitive network. This shift toward improved cognitive control should be particularly beneficial in depressed patients who suffer from rumination, negative anticipation, and excessive feelings of guilt and shame. — PRS

Proc. Natl. Acad. Sci. U.S.A. 110, 19597 (2013).

MATERIALS SCIENCE

Glassy Dynamics

In the glassy state, atomic or molecular motion is limited to localized regions within an overall disordered structure. As a material is cooled toward its glass transition temperature, there is a rapid increase in the viscosity, which is marked by a slowdown of the atomic or molecular motions that is stronger than one would predict from a simple Arrhenius law. One theory is that as the glassy material is cooled, there is an increase in the number of correlated molecules that need to move together. This leads to a temperature-dependent activation energy, $E(T) = \exp(\Delta/T)$, where Δ can be thought of as an energy barrier. Bauer *et al.* measured the third-order nonlinear dielectric susceptibility for four materials that included one "strong" glass former and two "fragile" ones. The latter are of particular interest, because the energy barrier Δ itself shows an excess component that is also temperature-dependent. Surprisingly, they find that there is a simple correlation between $E(T)$ and the number of correlated molecules, largely independent of the molecular interactions within each material. The formation of the glassy state and the rapid viscosity rise are thus primarily due to an increase in the number of atoms or molecules, whose motions couple together as the temperature is lowered. — MSL

Phys. Rev. Lett. 111, 225702 (2013).

ASTRONOMY

Bright Young Thing

Circinus X-1, a binary star system that includes a neutron star, is one of the brightest x-ray sources in the sky. Heinz *et al.* now report that this x-ray

binary is the youngest known yet. Data from the Chandra X-ray Observatory and from the Australian Telescope Compact Array, a radio telescope composed of six 22-m antennas, show that the neutron star is still within the supernova remnant in which it was born. A supernova remnant, the radiating material that is left after a star explodes at the end of its life, does not stay visible for very long. In this case, the age of the remnant constrains Circinus' age to less than 4600 years. Such a young age explains the system's rapid orbital evolution and highly eccentric orbit, which had been puzzling. There has not been enough time

all of them. Yang *et al.* used scanning tunneling spectroscopy to observe the evolution of the local density of states (LDOS) near a nonmagnetic impurity dopant (Cu) in the material $\text{Na}(\text{Fe}_{0.96-x}\text{Co}_{0.03}\text{Cu}_x)\text{As}$. The effect of a Cu impurity on LDOS appeared to be a pronounced enhancement near 2 meV, inside the superconductor gap. Such in-gap states near nonmagnetic impurities are consistent with the gap symmetry, where the electron and hole pockets of the Fermi surface have opposite signs of the gap function; the authors performed magnetization measurements to demonstrate that the Cu impurities are indeed nonmagnetic. Comparison to theory indicated that the results were incompatible with other proposed gap symmetries, which may have implications for the mechanism of superconductivity in these materials. — JS

Nat. Comm. **4**, 2749 (2013).

IMMUNOLOGY

Nanotoxoids

Some bacteria, such as *Staphylococcus aureus* and *Escherichia coli*, release toxins that punch holes into membranes to kill cells. Vaccines against such pore-forming proteins have generally used toxins that are inactivated by heat or chemicals to elicit a protective immune response. Although these treatments generate a safe vaccine,

they can destroy key antigenic epitopes, thus weakening the immune response. Hu *et al.* have taken an alternative approach. The authors coated polymer nanoparticles with membranes from mouse red blood cells. The particles then absorbed undenatured staphylococcus alpha-hemolysin (Hla) toxin into the membrane coating. The nanotoxoids were stable and could be taken up by mouse dendritic cells, the immune cells that normally process antigen. Unlike Hla, the nanotoxoid particles did not kill cells when injected into the skin of mice. The particles also triggered the production of antibodies to Hla in mice, but avoided provoking an autoimmune response to other membrane constituents. The immune response also protected animals from a lethal dose of Hla. After a single vaccination and two booster shots, 100% of Hla-treated mice survived. A nanoparticle-based immunoen-gineering approach could be used to develop a broad range of anti-toxin vaccines. — LC

Nat. Nanotechnol. **10**, 1038/
NNANO.2013.254 (2013).



yet for the orbit to be tidally circularized from the eccentricity it received in the explosion. Because the neutron star is known to have a low magnetic field, the young age also implies that neutron stars can be born with low magnetic fields or can easily become demagnetized. — MJC

Astrophys. J. **779**, 171 (2013).

PHYSICS

Mind the Gap

One of the basic characteristics of a superconductor is the energy needed to break up Cooper pairs, which form the superfluid flow in these materials. In conventional superconductors such as Nb, this energy, twice the size of the so-called superconductor "gap," does not depend on the momenta of the electrons forming the pair; however, in cuprates, for example, the gap disappears entirely at certain points in momentum space. The symmetry of the gap in iron-based superconductors is still under debate, and there are indications that it might not be the same for

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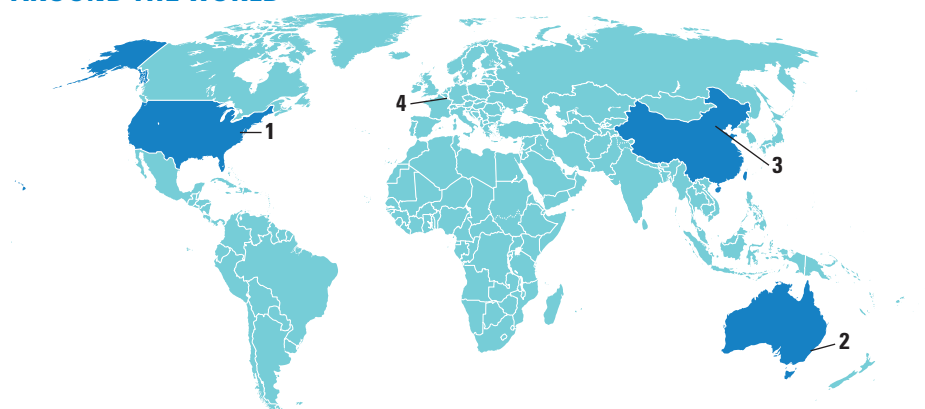
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AROUND THE WORLD



Silver Spring, Maryland 1

FDA Tackles Farm Antibiotic Use

In an effort to slow the evolution in farm animals of drug resistance, which could spread to humans, the U.S. Food and Drug Administration (FDA) last week announced changes in the use of antibiotics for livestock and poultry. It asked companies producing antibacterial drugs that it considers medically



Treat with care. U.S. will limit livestock drugs.

important to humans to voluntarily change their labels, removing claims of improved growth and feed efficiency.

These antibiotics would then require prescriptions from a veterinarian, who could approve use for treating sick animals or preventing disease in those considered “at risk.” FDA gives drug producers 3 years to make the change, and many companies support the plan.

The American Society for Microbiology applauded the move in an e-mail, calling it “a major step to address antibiotic resistance comprehensively.” But others doubt that voluntary action will be enough to protect public health. “The FDA may care whether companies call it growth promotion or disease prevention, but the bacteria do not,” said Kieve Nachman, an environmental health scientist with the Johns Hop-

kins Bloomberg School of Public Health in Baltimore, Maryland, in a statement. <http://scim.ag/FDAfarm>

Sydney, Australia 2

Port Plans Cast Shadow On Iconic Reef

The ecologically embattled Great Barrier Reef (GBR) may soon be host to one of the world’s largest coal ports—if a proposed construction project gets the go-ahead from Australia’s Marine Park Authority. Port construction would involve dredging at Abbot Point and dumping up to 3 million cubic meters of dredge spoils at an unspecified location within the GBR ecosystem, which is listed as a UNESCO World Heritage Site. Federal Environment Minister Greg Hunt approved the project on 10 December, but because it falls within the marine park boundary, a park authority permit is required.

“The development would clearly damage the health of the GBR,” asserts fisheries veterinarian Matt Landos of the University of Sydney. He says “lessons have not been learnt” from the 2010 to 2011 dredging south at Gladstone Harbour, which released toxic metals from the seabed, caused outbreaks of fish disease, and killed dolphins and turtles.

UNESCO warned earlier this year that further gas and coal development could put the reef on the “in danger” list. No decision had been made as *Science* went to press.

Beijing 3

China Scores Lunar Touchdown

After a 12-day voyage, China’s lunar probe Chang’e-3 landed at the Sea of Rains (also known as Mare Imbrium) in the moon’s northern hemisphere on 14 December. The event, broadcast live on national television,



Ready to rove. China’s Yutu rover on the moon.

was the first soft landing on the moon in 37 years.

“Everything has gone fantastically well,” says Wu Ji, director of the National Space Science Center in Beijing. “The lander kicked up much less dust than expected.” The Communist Party hailed the mission as a “new glory” of the Chinese people.

After 8 hours charging up with the spacecraft’s solar panels, the rover Yutu (Jade Rabbit) rolled down the lander’s ramp and onto the moon’s surface. “It was quite nerve-racking,” says Jia Yang, the spacecraft’s deputy chief engineer at the Beijing Institute of Spacecraft System Engineering. He says that Yutu is taking a “nap” now to avoid damage from the scorching lunar heat. It will then start its 3-month mission exploring the composition of the lunar surface and crust.

Luxembourg 4

Court Strikes Down GM Potato Approval

In another setback for genetically modified (GM) crops in the European Union, the General Court last week annulled the authorization to grow and sell a GM potato called Amflora. The ruling won’t affect the potato’s cultivation or sale, because its manufacturer withdrew from the European market last year. But it may hinder the approval of a GM maize variety, which the European Commission tried to drive forward last month after a lengthy deadlock.

BY THE NUMBERS

80% Proportion of data from publicly funded research that’s inaccessible 20 years after publication thanks to problems such as old e-mail addresses and obsolete storage devices, according to a study in *Current Biology* this week. <http://scim.ag/datalost>

THEY SAID IT

"They should make at least a fraction of what some Wall Street trader makes."

—Billionaire entrepreneur Yuri Milner at an award ceremony for winners of the Breakthrough Prizes in Physics and Life Sciences, where he announced a new \$3 million prize for mathematics.

Random Sample



A Makeover for Big Screen Dinosaurs

The world of Patchi the Pachyrhinosaurus is no *Jurassic Park*. The computer-animated protagonist of the new movie *Walking with Dinosaurs* inhabits a fictional land informed by recent science. That's the verdict from paleontologist Steve Brusatte of the University of Edinburgh in the United Kingdom, who was among a handful of dino consultants on the film.

As a budding scientist, Brusatte was enraptured by the original *Walking with Dinosaurs*, a 1999 BBC miniseries that inspired the current movie reimaging. "When I look at the film, I'm very happy with how they portrayed the dinosaurs," he says. "The point of the film isn't to present new science, but it incorporated new science to tell the story."

That story follows Patchi as he grows from a punchy underdog of a hatchling to an unlikely leader of his migrating herd. Viewers accustomed to the traditional "scaly lizard" image may find the feathery meat-eating dinosaurs jarring. And the volatile environment of the Late Cretaceous, just a few million years before the dino-obliterating asteroid, adds to the drama, as characters struggle to survive in a world of wildfires, volcanic eruptions, and fluctuating temperatures and sea levels. The parental care lavished upon young Patchi and the communal lifestyle of his herd also find support in the fossil record, Brusatte says. All in all, sticklers for scientific accuracy will be pleased, he predicts—provided they can "get beyond the fact that these things are talking."



Hot potato. A 2010 protest in Germany against genetically modified potatoes.

The potato is engineered to produce a starch used in paper pulp, glue, and animal feed. Based on advice from the European Food Safety Authority, the European Commission authorized it in March 2010. But it did not submit the final decision to the relevant committee of member states' representatives. Hungary challenged that decision in court, with support from Austria, France, Luxembourg, and Poland.

Environmental groups are now calling on the commission to withdraw its proposal to approve Pioneer's GM maize 1507, which they say followed a similarly flawed procedure. A commission spokesman says its legal services will analyze the ruling and its possible consequences.

<http://scim.ag/GMannul>

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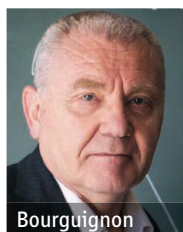
Three Q's

The European Commission announced this week that French mathematician **Jean-Pierre Bourguignon** will be the new president of the European Research Council (ERC), the European Union's main funding agency for basic research. After 19 years directing the Institute of Advanced Scientific Studies in Paris, Bourguignon joins the agency at the dawn of the European Union's new 7-year research program, Horizon 2020, which will

dramatically increase ERC's budget.

Q: Why did you decide to take the job?

J.-P.B.: [One reason] is that I'm a very convinced European. If you ask scientists what the greatest success for science in the European Union is, many will say: the ERC. So being involved in that was very natural to me.



Bourguignon

Q: Will you make changes in ERC's existing grants?

J.-P.B.: There have been two rounds of Synergy Grants [a grant for small interdisciplinary groups of researchers that started in 2012]. What I have seen from the outside doesn't give me a very good impression of it. ... So this program needs to be reviewed very, very carefully.

Q: What do you tell those countries that have fared poorly in the competition for grants?

J.-P.B.: The answer isn't obvious at all. Sometimes the level of science [in a country] just isn't high enough; then that has to be addressed in itself. But we must absolutely not go to the situation where every country gets out of the ERC what they put into it—the principle of *juste retour*, as the French call it. That would be the worst idea.

Extended interview at <http://scim.ag/BourgERC>.

Science LIVE

We will return on Thursday, 9 January, at 3 p.m. EST for a live chat with experts on **preserving threatened large carnivores to stabilize ecosystems**. <http://scim.ag/science-live>

PALEONTOLOGY

Mega-Eruptions Drove the Mother of Mass Extinctions

SAN FRANCISCO, CALIFORNIA—After 20 years of trying, researchers have finally convicted massive volcanic eruptions in Siberia as the culprit in the greatest of all mass extinctions, one that destroyed 90% of marine species on the planet. The key evidence—reported here last week at the fall meeting of the American Geophysical Union—came from geochronologists applying the latest dating techniques to both the basalt from the eruptions and the rock encasing fossils of creatures that went extinct about 252 million years ago.

“I’m excited by the very clean-looking dating,” says paleontologist Paul Olsen of the Lamont-Doherty Earth Observatory in Palisades, New York. “It shows you could in fact have the Siberian eruptions cause the mass extinction.” Now, the question is which of the many possible ways that the volcanism could have wiped out species was actually at work.

Suspicion fell on the Siberian Traps—a vast volcanic landscape—more than 2 decades ago because of its enormous size and its age. In one of the greatest volcanic outbursts in Earth’s history, these eruptions carpeted a Western Europe-sized area of Siberia with several million cubic kilometers of basalt. Geochronologists measuring time by the slow but steady radioactive decay of uranium-238 to lead-206 in tiny zircon crystals initially found the eruptions lasted about 2 million years and took place roughly at the time of the mass extinction: the Permian–Triassic (P–T) boundary, when the Permian period ended and the Triassic period began. But there was no way to tell for sure whether the eruptions came before the extinction and thus could have caused it.

Over the past decade, geochronologists continued to improve their craft. They developed a way to eat away the parts of zircon crystals that had spilled some of their cargo of uranium and lead, resetting the radiometric clock. They improved

their methods for calibrating the isotope measurements. And they dated rock from both the eruptions and the extinction in a single lab using the same techniques.

Geochronologists Seth Burgess and Samuel Bowring of the Massachusetts Institute of Technology (MIT) in Cambridge applied these improvements to rock from the traps and to the rock containing the P–T extinction boundary from Meishan, China. The result: “We can say that, yes, magmatism preceded the mass extinction,” Burgess says. The group’s best age for the initial eruptions is 252.28 million years. They dated the beginning of the extinction at 251.941 million

warming, Shen reported. But the rocks, from Guangxi province in South China, show the warming came just after the extinction, ruling out a role in the die-off.

Shen remarked that the exceptionally detailed South China fossil record suggests that the extinction was “very short, only a few thousand years,” a rapidity that supports other potential kill mechanisms including acid rain from sulfur dioxide emissions. Atmospheric modeler Benjamin Black of MIT and his colleagues reported that injecting 1.5 billion tons of volcanic sulfur dioxide into a computer model of the Permian atmosphere acidified rain across the Northern Hemisphere to pH 2, about that of lemon juice. That would have been disastrous for exposed vegetation and every animal that depended on it, he noted.

The volcanism may also have touched off toxic coal fires. Geologist Stephen Grasby of the Geological Survey of Canada at the University of Calgary and colleagues found microscopic carbonaceous particles in shale rock deposited in the Canadian High Arctic just before the P–T boundary. The particles bear a striking resemblance to the fly ash spewed by coal-burning power plants. As it erupted, the magma that formed the Siberian Traps is known to have pierced coal deposits.

Grasby and his colleagues think the result must have been a vast, subterranean, coal-fired inferno that belched metal-bearing ash into the stratosphere, where the toxic debris

sifted across the Northern Hemisphere. In fact, Grasby and colleagues found spikes in mercury in each ash-containing layer and far higher mercury abundances at the P–T boundary itself.

Now that geochronologists have refined their dating tools, they hope to test suspicions that volcanism was at work in other mass extinctions. Dates for the huge eruptions at the opening of the Atlantic Ocean 201 million years ago and the mass extinction that cleared the way for the dinosaurs overlap, but their order is yet to be determined (*Science*, 21 December 2012, p. 1522). And several other possible pairings await. **—RICHARD A. KERR**



Weapons and victim. Waterfalls grace two lava flows of the Siberian Traps, remains of vast eruptions that snuffed out Permian life like the coil-shelled ammonite (inset).

years ago and its end at 251.880 million years ago. Uncertainties ranged from 0.031 million to 0.110 million years. That puts the volcanic and extinction events in the proper order and close enough to be cause and effect.

Now, researchers can focus on possible kill mechanisms. At the meeting, paleontologist Shuzhong Shen of the Nanjing Institute of Geology and Palaeontology in China said one suspect should be dropped: sudden global warming caused by carbon dioxide pouring from the traps’ eruptions. Analyses of temperature-sensitive oxygen isotopes in sediments deposited around the time of the extinction reveal a whopping 8°C to 10°C

ASTRONOMY

Money Woes Cloud Future of Workhorse U.S. Telescopes

Five federally funded optical and radio telescopes in the United States could be forced to shut down over the next 3 years because of budget cuts by the National Science Foundation (NSF). The anticipated closures—all but certain unless alternate sources of funding materialize—will leave astronomers at institutions that lack their own telescopes out in the cold. No publicly accessible optical telescopes, and just one public-access radio telescope, would be left in the continental United States.

The decision has sparked widespread dismay among astronomers, who say the loss will severely hurt students and early-career scientists, especially at small and mid-sized institutions. But NSF officials say the agency has no choice but to divest itself of these five telescopes if it is to operate new telescopes expected to come online in the next several years, including the Advanced Technology Solar Telescope (ATST)—scheduled for first light by 2018—and the Large Synoptic Survey Telescope (LSST), a \$665 million sky-surveying instrument to be built in Chile starting next year.

Optical astronomers would lose three telescopes operated by the National Optical Astronomy Observatory (NOAO) at Kitt Peak in Arizona: a 2.1-meter telescope, the 4-meter Mayall telescope, and the 3.5-meter Wisconsin-Indiana-Yale-NOAO (WIYN) telescope, which is supported by those three universities in partnership with NOAO. The radio telescopes to be shuttered are the 100-meter Robert C. Byrd Green Bank Telescope (GBT) in Green Bank, West Virginia, and the Very Long Baseline Array (VLBA), a series of 10 smaller antennas spread between Hawaii and the Virgin Islands, both operated by the National Radio Astronomy Observatory (NRAO).

Their futures were thrown into doubt last year, when a panel NSF had appointed to review its astronomy portfolio recommended that the agency stop funding them to realize much-needed cost savings. Last month, NSF's astronomy division head, James Ulvestad, informed the Astronomy and Astrophysics Advisory Committee (AAAC)—a panel that guides decision-making in astronomy across the federal government—that his division

was in the process of implementing the recommended cuts.

"I am extremely dismayed at this choice," says M. Virginia McSwain, an astronomer at Lehigh University in

Ulvestad acknowledges that the cuts will cause pain but argues that they were the best way to limit the damage of a flat budget. NSF's astronomy division is now funded at \$235 million a year. Over the past few years, Ulvestad says, the division has been forced to reduce its grants to researchers to keep its facilities operational. Already committed to beginning construction of the LSST, as well as operating the ATST in Hawaii, which is now under construction, the agency could not continue funding all existing telescopes

	Telescopes	Location	Operated by	Status
Optical	2.1-m telescope at Kitt Peak	Kitt Peak, Arizona	National Optical Astronomy Observatory	Closing July 2014
	4-m Mayall telescope	Kitt Peak, Arizona	National Optical Astronomy Observatory	To shut down by 2017, unless supported by Department of Energy
	3.5-m WIYN telescope	Kitt Peak, Arizona	National Optical Astronomy Observatory	Operations to end by 2017; universities being solicited for support
Radio	Green Bank Telescope	Green Bank, West Virginia	National Radio Astronomy Observatory	To shut down by 2017; efforts ongoing to find new funders
	Very Long Baseline Array	Hawaii to U.S. Virgin Islands	National Radio Astronomy Observatory	To shut down by 2017; efforts ongoing to find new funders

Dimming prospects. Belt tightening at the National Science Foundation threatens to close five of the six publicly accessible telescopes in the continental United States.

Bethlehem, Pennsylvania, whose graduate students routinely use the Kitt Peak telescopes for their research. "This will have major negative effects on training." Students and researchers at institutions like Lehigh, McSwain says, would have to travel to public facilities in Hawaii or Chile to conduct optical observations, trips some could not afford. "If I did receive observing time in Chile, do you think I would send a graduate student there? No, of course not," she says. "This is not just about the loss of jobs for people who manage these five telescopes. It's about the loss of a generation of astronomers."

Radio astronomers are similarly dismayed. The GBT "is an incredibly valuable instrument," says Scott Ransom, an NRAO astronomer, pointing to the many pulsars it discovered in recent years. He notes that the GBT, though commissioned in 2000, was equipped to make observations at the high-frequency end of the radio band only in 2009. "The sad thing to me is that just as the GBT is starting to do really great science, they're getting ready to pull the plug."

without drastically undermining support for research grants. And that's why, Ulvestad says, the agency is winding down support for telescopes that the 2012 portfolio review identified as scientifically less important than others. "We don't want to just keep cutting individual grants to fund facilities," Ulvestad says. "Facilities will have to take a cut, too." Divesting the five telescopes will generate a net savings of about \$20 million a year.

New sources of funding could still turn up to save some of the facilities. The Mayall 4-meter telescope at Kitt Peak could be rescued by the Department of Energy (DOE), says NOAO Director David Silva. NOAO is working with DOE's Lawrence Berkeley National Laboratory to develop a spectrograph known as the Dark Energy Spectroscopic Instrument (DESI), which researchers plan to install on the Mayall for an ambitious project aimed at measuring dark energy. The big if is that DOE still hasn't committed to funding DESI, although Silva is optimistic. He is also hopeful that ongoing efforts to expand the partnership behind the 3.5-meter WIYN telescope

will succeed in keeping it afloat.

Similar efforts are on at NRAO to find partners to run the GBT and the VLBA. West Virginia University, for instance, recently stepped in to support the GBT with \$1 million over 2 years. But that's a small fraction of the annual \$8 million needed to operate the facility.

Ulvestad says that NSF would consider partnerships to be viable only if they reduce NSF's costs by 50%. "The VLBA costs about \$6 million to operate," he says by way of example. "That means a partnership that

only cuts the NSF cost by \$1 million doesn't really help us."

Angela Speck, an astronomer at the University of Missouri, Columbia, and a member of AAAC, says it's imperative

Slated. Kitt Peak's Mayall telescope could close by 2017.

that a solution be found. "More than half the optical-infrared astronomers in the U.S. work at institutions without their own access to telescope facilities," she says. "Consequently, they rely on NOAO for both research and training of the next generation of observational astronomers." If only "large

international consortia" are supported, she says, the future health of U.S. astronomy could be in jeopardy.

—YUDHIJIT BHATTACHARJEE



SCIENCE FUNDING

U.S. Budget Deal Offers Researchers Some Sequester Relief

A 2-year budget agreement approved this week by Congress brings the federal government one step closer to temporarily removing the universally reviled, across-the-board cuts known as sequestration. But the agreement leaves the U.S. scientific community a long way from the sustained growth in federal research spending it wants. And it could be nearly a month before scientists learn what the deal means for specific research agencies.

Announced on 10 December, the agreement marks a cease-fire between Republicans and Democrats in the ongoing war over how to reduce the federal deficit, which in 2013 was \$680 billion after topping \$1 trillion for several years. Crucial for scientists, it provides \$22 billion more in 2014 than what would have been available under sequestration for the slice of the federal budget that supports all civilian research. That slice, called nondefense discretionary spending, will grow by 4.7%, to \$491 billion, in the 2014 fiscal year, and to \$492 billion in fiscal 2015. (That is \$9 billion more in 2015 than specified under sequestration.)

Budget analysts at AAAS (the publisher of *Science*) estimate that the agreement

could result in an overall 2.7% boost, to \$136 billion, in what the government spends on research and development in 2014. (President Barack Obama had proposed \$144 billion for research and development in his 2014 budget request to Congress.) Sequestration, which took a 5% bite out of most research agency budgets earlier this year, remains a threat, but not before 2016.

Academic and industry lobbyists say the

automatic cuts, created by a 2011 law aimed at shrinking the federal deficit over the next decade, have impeded research aimed at improving the nation's health, prosperity, and security. So they have been unanimous in praising what Hunter Rawlings, president of the Association of American Universities, called "a modest but important easing of sequestration."

"As cynical and pessimistic as I've become about the process over the 29 years I've been doing this, there are some glimmers of hope," says David Moore of the Association of American Medical Colleges in Washington, D.C. "One is that we'll be able to be more certain about spending for the next 2 years."

Moore is referring to the budget agreement's green light to spending panels in both the House of Representatives and the Senate to craft budgets for individual agencies. The work, parceled out to 12 appropriations subcommittees, is supposed to be a transparent process that lasts months. And earlier this year, the panels passed several such bills covering important research agencies (see table).

But political gridlock has

Where Science Budgets Stand			
Agency or program	2013 budget	2014 House mark	2014 Senate mark
National Institutes of Health	29.15	none	30.95
National Science Foundation	6.884	6.994	7.425
<i>Research</i>	5.543	5.676	6.018
<i>Education</i>	0.833	0.825	0.880
DOE Office of Science	4.662	4.653	5.152
Advanced Research Projects Agency - Energy	0.252	0.050	0.379
National Institute of Standards and Technology	0.769	0.784	0.947
NASA	16.9	16.6	18.0
<i>Science Office</i>	4.78	4.78	5.15
Agricultural Research Service	1.019	1.107	1.123
National Oceanic and Atmospheric Administration R&D	0.769	0.784	0.947

A budget at last? The new budget agreement provides a way to reconcile different starting points for each house of Congress.

made it impossible in recent years to turn those bills into law. Instead, legislators increasingly have turned to massive spending bills, adopted at the 11th hour, that paper over policy disagreements by treating most agencies alike.

This year, for example, that lack of comity created a situation in which House appropriators worked to divvy up a pot of money for 2014 that was \$91 billion smaller than what their Senate counterparts were using. That's because Republican House leaders had factored in sequestration, while Democratic Senate leaders assumed it would be eliminated.

The budget agreement splits the difference. "The final [budget] numbers came in higher than a lot of people would have predicted when they started negotiating," Moore notes. "So it does give the appropriators some latitude in restoring the cuts" to the National Institutes of Health (NIH) and other research agencies. Overall, the agreement, which also covers defense programs, will increase discretionary spending by \$63 billion over a decade. To raise those funds, it creates a few revenue "enhancements," such as new fees on airline travel. It also produces \$23 billion in additional savings by extending the overall budget agreement by 2 years, through 2023.

Congressional staffers are now hammering out the spending details. Legislators could simply adopt the midpoint between the House and Senate versions for the thousands of programs described in the various spending bills. But because budget bills are a critical way for members to advance their priorities, some agency programs are likely to do better than others.

It's also possible that Congress could approve detailed spending plans for some agencies while letting others be covered in a single bill, called an omnibus, which would give the same percentage increase to every agency it covers. That outcome seems likely for NIH, whose parent spending bill is always contentious.

Legislators are working with a tight deadline: Agencies are now operating under a temporary agreement, called a continuing resolution (CR), which has frozen spending at 2013 levels. The CR expires on 15 January 2014, however, and without a final spending agreement the government would have to shut down. If the process breaks down, Congress could extend the CR for weeks or months at current spending levels. That outcome is something neither party wants, and that scientists hope doesn't come to pass.

—JEFFREY MERVIS AND DAVID MALAKOFF



MATERIALS SCIENCE

Amid Superconductor Debate, Clash of Physics Titans Resumes

Robert Laughlin is back with a vengeance. After a decade away from physics, the Nobel laureate at Stanford University in Palo Alto, California, argues in a pair of soon-to-be published papers that most physicists' basic assumptions about the origins of high-temperature superconductivity—the ability of certain materials to carry electricity without resistance at still frigid, but unusually high temperatures—are wrong. Instead, Laughlin argues, the biggest mystery in condensed matter physics can be explained starting from the conventional theory of metals, a tack most theorists abandoned decades ago.

Laughlin has spent recent years writing popular science books and had a turbulent tenure from 2004 to 2006 as the president of the Korea Advanced Institute of Science and Technology in Daejeon. He declined to answer questions about his papers, which are in press at *Physical Review Letters* and *Physical Review B*, saying they speak for themselves. But he clearly views his work as the jolt that will pull the superconductivity field out of the quagmire it has languished in for years (*Science*, 17 November 2006, p. 1072). "What's playing out here is something that happens rarely in physics," Laughlin writes in an e-mail. "A deeply rooted consensus belief [is being] smashed by one man using logic."

As much as that jolt is clearly needed, other physicists say that Laughlin has ignored experimental evidence that undermines his ideas. And some view Laughlin's papers as a continuation of a long-running feud with another Nobel laureate, Philip Anderson of Princeton University, who has advocated a very different explanation of high-temperature superconductivity. "I think that what's

Back in the fray. Robert Laughlin, seen here in 1998 celebrating his Nobel Prize, had been out of theoretical physics for several years.

happened is that Phil has staked out a really extreme position, and now that Bob is coming back into the business he's trying to stake out the opposite extreme," says Michael Norman, a theorist at Argonne National Laboratory in Lemont, Illinois.

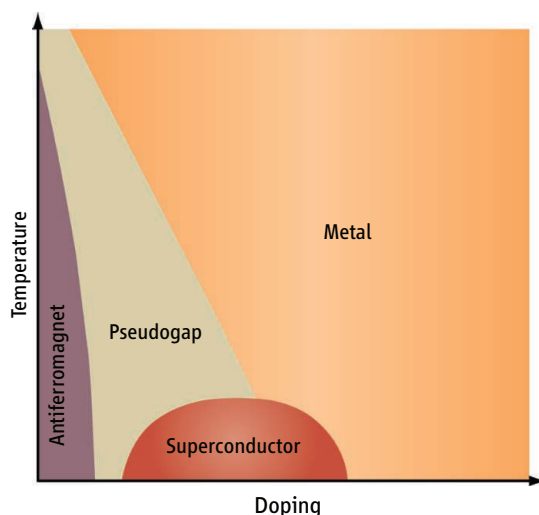
Laughlin takes what might be called a radically conventional approach to high-temperature superconductivity. Ordinary superconductors are metals such as lead or niobium. When the materials are cooled to within a few degrees of absolute zero, the electrons within them form loose pairs, bound by vibrations of the array of positively charged ions between them, that can glide unimpeded through the metal.

But that vibrational glue isn't strong enough to explain how some compounds of copper and oxygen become superconductors at temperatures as high as 138 kelvin. In bismuth strontium calcium copper oxide, yttrium barium copper oxide, and other "cuprates," copper and oxygen atoms form planes with the other atoms between them. Within each plane, the coppers arrange themselves in squares and the oxygens sit in between neighboring copper atoms. In a metal, each copper atom would contribute one mobile electron to the material. In the cuprate compounds, however, the electrons get stuck, one to a copper atom, in a traffic jam known as a Mott insulator. Researchers "dope" the compound with extra oxygen, which absorbs some of the copper electrons, breaking the jam and somehow triggering superconductivity.

Twenty-seven years after high-temperature superconductivity was discovered, theorists still don't agree on how that happens. In the copper-and-oxygen planes, neighboring electrons are magnetized in opposite directions in a pattern known as antiferromagnetism. Some theorists argue that ripples in this pattern provide a glue to bind electron pairs, as in traditional metal superconductors. But others, such as Princeton's Anderson, argue that no glue is needed and that the strong repulsion among the electrons alone create pairing.

Laughlin dismisses both views and starts anew with the basic theory of metals. Electrons normally repel one another mightily, but the theory holds that in metals, electrons behave as if they do not interact. Theorists explain that situation by envisioning noninteracting electrons and ramping up the forces among them. The interactions forge noninteracting "quasiparticles"—motions of many electrons have the same charge as individual electrons but different masses and magnetism and a finite lifetime.

Laughlin argues that the cuprates must be approached in the same way. He begins with quasiparticles residing on copper and oxygen planes. By adjusting parameters such as the strength with which the quasiparticles repel each other, the rate at which they hop from atom to atom, and the magnetic forces among them, he can theoretically reproduce how the behavior of the cuprates changes as a function



Multifarious. A high-temperature superconductor exhibits different behaviors or phases depending on its temperature and how much oxygen it's doped with.

of doping and temperatures (see diagram). For example, he claims that the distinctive "pseudogap" phase, in which electrons seem to pair but not flow freely, is actually a pattern of currents known as a d-density wave—even though that pattern has been searched for and never found.

Laughlin says that the new work is primarily a challenge to the idea of the Mott insulator, which he insists rests on a conceptual misunderstanding. The Mott state cannot be derived from noninteracting quasiparticles by turning on interactions, he argues in a draft of his *Physical Review B* paper, and "therefore does not exist."

But others contend that Laughlin has

simply not kept up with the science. Tin-Lun Ho, a theorist at Ohio State University, Columbus, says the Mott insulator state has been demonstrated in experiments that simulate crystals with cold atomic gases. And Patrick Lee, a theorist at the Massachusetts Institute of Technology in Cambridge, says the values of the parameters Laughlin uses to theoretically reproduce the cuprates' known phases clash with experimental values.

In spite of their reservations, many theorists say they're glad to see Laughlin back in the business. "We all love Bob," Norman says. "He's larger than life."

Anderson does not share that enthusiasm. In a 2004 issue of the humor magazine *Annals of Improbable Research*, Laughlin published a 13-page poem riffing on Henry Wadsworth Longfellow's *The Song of Hiawatha* in which he seems to portray himself as the innocent hero led astray by an unnamed figure who is clearly Anderson.

When asked about Laughlin's papers, Anderson said he was too busy preparing for his 90th birthday party last week to immediately address them in detail. But, he noted, Laughlin "does not seem to have read or understood any of my rather careful explanations of the Mott physics involved in the cuprates." Anderson acknowledged that he was glad Laughlin left the field and is distressed at his return: "Bob—a.k.a. Hiawatha—has gone nutty."

—ADRIAN CHO

ARMS CONTROL

Scientists Campaign Against Killer Robots

Your mission: Persuade the global military industrial complex to forgo a class of weapons that could transform warfare in the 21st century. A quixotic quest, you say? Noel Sharkey would disagree. In a provocative op-ed in *The Guardian* in 2007, the computer scientist at the University of Sheffield in the United Kingdom wrote that before long, robotic weapons would be delegated the authority to decide which human targets to kill. For many, the warning may have conjured fictional cyborgs, but for some artificial intelligence experts, it echoed a real fear of automated weapons relying on nothing but algorithms to tell the difference between combatants and civilians.

Thanks to the efforts of Sharkey and other scientist-campaigners, the dawn of self-

directed robotic killing machines may yet be prevented. Meeting last month in Geneva, the 117 state parties to the Convention on Certain Conventional Weapons (CCW) agreed to examine the regulation of autonomous weapons. CCW provisions already outlaw or regulate land mines, firebombs, cluster munitions, and blinding lasers. The prospect of adding roboweapons to the list "is no longer a fantasy," says Sharkey, chair of a nongovernmental group called the International Committee for Robot Arms Control (ICRAC). "We have taken a major step toward stopping these odious weapons from being deployed."

Just as the tank, the submarine, and the atomic bomb rewrote the rules of war in the 20th century, autonomous weapon systems

are one of a handful of technologies heralded as potential game-changers in this century's conflicts. "Military are working on ever more autonomous systems not because they're neat, not because they're cool, but because people see battlefield advantage," says Peter Singer, director of the Center for 21st Century Security and Intelligence at the Brookings Institution in Washington, D.C.

The U.S. Department of Defense (DOD) laid out a 25-year vision for unmanned systems in a December 2007 report that included a call for robots that autonomously locate and destroy targets. More alarming for Sharkey and his fellow campaigners, DOD in November 2012 issued a policy directive that not only affirmed that robotic weapons may be directed against "materiel

CREDIT: AAAS/SCIENCE

targets,” but also implied that humans could be targeted—if top defense officials signed off on “formal development.”

“Without fanfare, the world had its first openly declared national policy for killer robots,” wrote ICRAC’s Mark Gubrud, a former research associate at the Program on Science and Global Security at Princeton University, in the 20 September issue of the *Bulletin of the Atomic Scientists*. Gubrud was one of the first observers to sound the alarm when in 2002, he and fellow physicist Jürgen Altmann of Technische Universität Dortmund in Germany called for a killer robot ban to prevent a future arms race. DOD’s 2012 directive, Gubrud wrote, “in effect overrides longstanding resistance within the military ... and signals to developers and vendors that the Pentagon is serious about autonomous weapons.”

The United States and other countries have long embraced unmanned systems—primarily drones, which DOD and the CIA have deployed on hundreds of missions to kill members of the Taliban, al-Qaida, and other terrorist organizations. So far, however, a human operator always calls the shots, and U.S. defense officials have been lukewarm about allowing robots to make the decision to kill.

One critical objection is that lethal autonomous weapons are unacceptable under the Law of Armed Conflict (LOAC). Rooted in ancient traditions and strengthened after the devastation visited on civilians during World War II, LOAC mandates that armed forces constrain their activities to military necessities and distinguish combatants from noncombatants. Military lawyers fretted that robots capable of automatically killing women and children would put the United States out of compliance with LOAC. But they do not see the same problem for robotic weapons that target things rather than people. A comparable distinction is made between antipersonnel land mines and those that target tanks. Only the former are banned—by the 1997 Ottawa Treaty—on the grounds that they target people indiscriminately.

Gubrud warns that the DOD directive approves the development of weapons

systems it calls “semi-autonomous” but which have fully autonomous targeting capabilities, including “lock-on-after-launch” missiles that are effectively hunter-killers. An example is the U.S. Navy’s Long Range Anti-Ship Missile, which will autonomously search for and attack ships from launch points hundreds of kilometers away. Even so, a Pentagon official earlier this year told Gubrud that “there are no completely autonomous systems in the pipeline or being considered.”



On the campaign trail. Noel Sharkey has fought for a roboweapons ban.

To Sharkey, the military’s aspirations to use lethal automated weapons as what he calls “force multipliers” run aground on a key ethical concern he articulated in *Science* 5 years ago (19 December 2008, p. 1800): “[N]o computational system can discriminate between combatants and innocents in a close-contact encounter. Computer programs require a clear definition of a noncombatant, but none is available.” Some computer experts assert that future sophisticated artificial intelligence systems, or “strong AI,” may someday equal or exceed human capabilities. The risk is that humans will lose control, Gubrud contends. “Stupid robots are dangerous, but smart robots are even more dangerous,” he says. “The most dangerous of all may be those that are right in between.”

The seeds of a grassroots scientific opposition were planted in September 2009, when Sharkey, Altmann, and two philosophers founded ICRAC. The organization’s mission statement declares, among other things, that “machines should not be allowed to make the decision to kill people.” Experts in robotics, AI ethics, and

law joined the cause. Sharkey, known in the United Kingdom as host from 1998 to 2004 of the popular BBC TV series *Robot Wars*, dove into campaigning for ICRAC full time.

In April, ICRAC banded together with Human Rights Watch and other groups—now 47 organizations from 22 countries—to form the Campaign to Stop Killer Robots. The movement gained momentum a few weeks later when Christof Heyns, U.N. special rapporteur on extrajudicial, summary, or arbitrary executions, called on the United

Nations Human Rights Council to “press all States for moratoria on the testing, production, assembly, transfer, acquisition, deployment and use of [lethal autonomous robots], until a governing framework is agreed.”

Sharkey and his comrades, meanwhile, set out to convince CCW signatories to take up the cause. The United States was key, Sharkey says, and he had reason to be hopeful. He says U.S. military brass respect ICRAC’s position because it is limited and specific. “We’re

only against the kill function,” Sharkey says. On 18 October, the U.S. delegation to the United Nations told him and his colleagues that they would support a CCW mandate to study autonomous weapons.

Speaking to a packed room at the CCW meeting in Geneva last month, Sharkey made an impassioned plea about the dangers of autonomous weapons. Any of the 117 delegations could have vetoed his proposal for a formal review. “This was nail-biting stuff,” Sharkey says. On the next day, 15 November, CCW parties unanimously agreed to convene a review meeting in May.

“This is a momentous opportunity to get states on the record and behind a ban,” said ICRAC member Heather Roff, a political theorist at the University of Denver, after the CCW decision. Over the next year, Sharkey says, he and other scientist-campaigners will try to persuade nations to adopt a protocol to CCW that would prohibit the development, production, and use of fully autonomous weapons—and in that way, keep the genie in the bottle.

—RICHARD STONE

Swine Flu Connection Provides Clues About Narcolepsy

The swine flu pandemic of late 2009 had a peculiar aftereffect in parts of Europe: a spike in children being diagnosed with narcolepsy, an incurable sleep disorder with symptoms including bouts of overwhelming daytime sleepiness. Researchers eventually linked a vaccine widely used to stave off the H1N1 flu virus to a small but increased risk of narcolepsy in children and teens. The vaccine, Pandemrix, used only in Europe, apparently triggered the disorder in roughly one out of 16,000 recipients in Finland. Sweden, Ireland, and England also found an increased risk, though not as dramatic as Finland's.

Now, scientists have a clue to why—one that points to a new understanding of narcolepsy itself, they report this week in *Science Translational Medicine*. They found that patients with the disease have immune

maker, GlaxoSmithKline, funded research to investigate what, if anything, in the vaccine could trigger the disease. Narcolepsy researcher Emmanuel Mignot of Stanford University, who received one of the grants, already suspected that rogue immune cells might contribute to narcolepsy. In 2009, Mignot and colleagues had found that almost all narcolepsy patients have a particular human leukocyte antigen (HLA) type. HLA molecules present antigens—pieces of foreign proteins—to T cells, which then coordinate the immune system's attack. The process helps the immune system distinguish between self and foreign cells, and certain HLA types are associated with autoimmune diseases such as type 1 diabetes and multiple sclerosis. But there is no obvious sign of immune attack in narcoleptic brains.

In the new work, Mignot, Stanford

after receiving the Pandemrix vaccine with T cells from the patients' healthy siblings, all of whom had the same HLA type and were also vaccinated. The patients' T cells reacted to the hypocretin epitopes, while their siblings' cells did not.

They then wondered whether the H1N1 virus itself might provoke the same immune reaction. A computer search turned up certain stretches of a key part of the H1N1 virus, the hemagglutinin protein, that "looked strikingly the same" as the hypocretin epitopes, Mignot says. Sure enough, in lab tests the viral protein fragment activated hypocretin-reactive immune cells—strong evidence that narcolepsy could be due to a process known as molecular mimicry, in which protein fragments from an invading virus or other pathogen prime the immune system to react to native proteins with a similar molecular structure.

The results are "exactly what we've been waiting for," says vaccine expert Hanna Nohynek of the National Institute for Health and Welfare in Helsinki, who was part of the team that first identified the increased risk of narcolepsy in children who received Pandemrix.

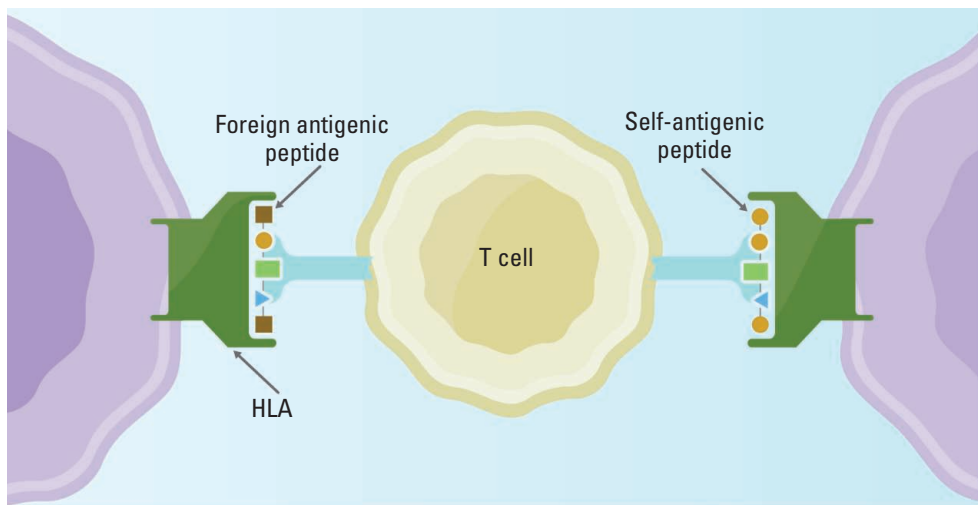
But molecular mimicry alone can't explain the whole mystery. It's not clear how an immune response to hypocretin could lead to the destruction of the neurons that produce it, as reactive cells in the blood would not necessarily reach these brain cells.

And it is still unclear whether H1N1 flu alone might trigger narcolepsy. Mignot and his colleagues have reported an increase in the disorder among unvaccinated Chinese

children who had the virus. But researchers in South Korea and Europe have found no increased risk of the disorder in people who had swine flu.

"There's plenty of work to do," says vaccine expert Steven Black of Cincinnati Children's Hospital Medical Center in Ohio, who is investigating possible links between H1N1 vaccines and narcolepsy. But the work "is the first mechanistic explanation of the disease," which should help researchers home in on the factor in Pandemrix that might have caused problems. Identifying that, he says, "is a first step" to being able to make even safer vaccines.

—GRETCHEN VOGEL



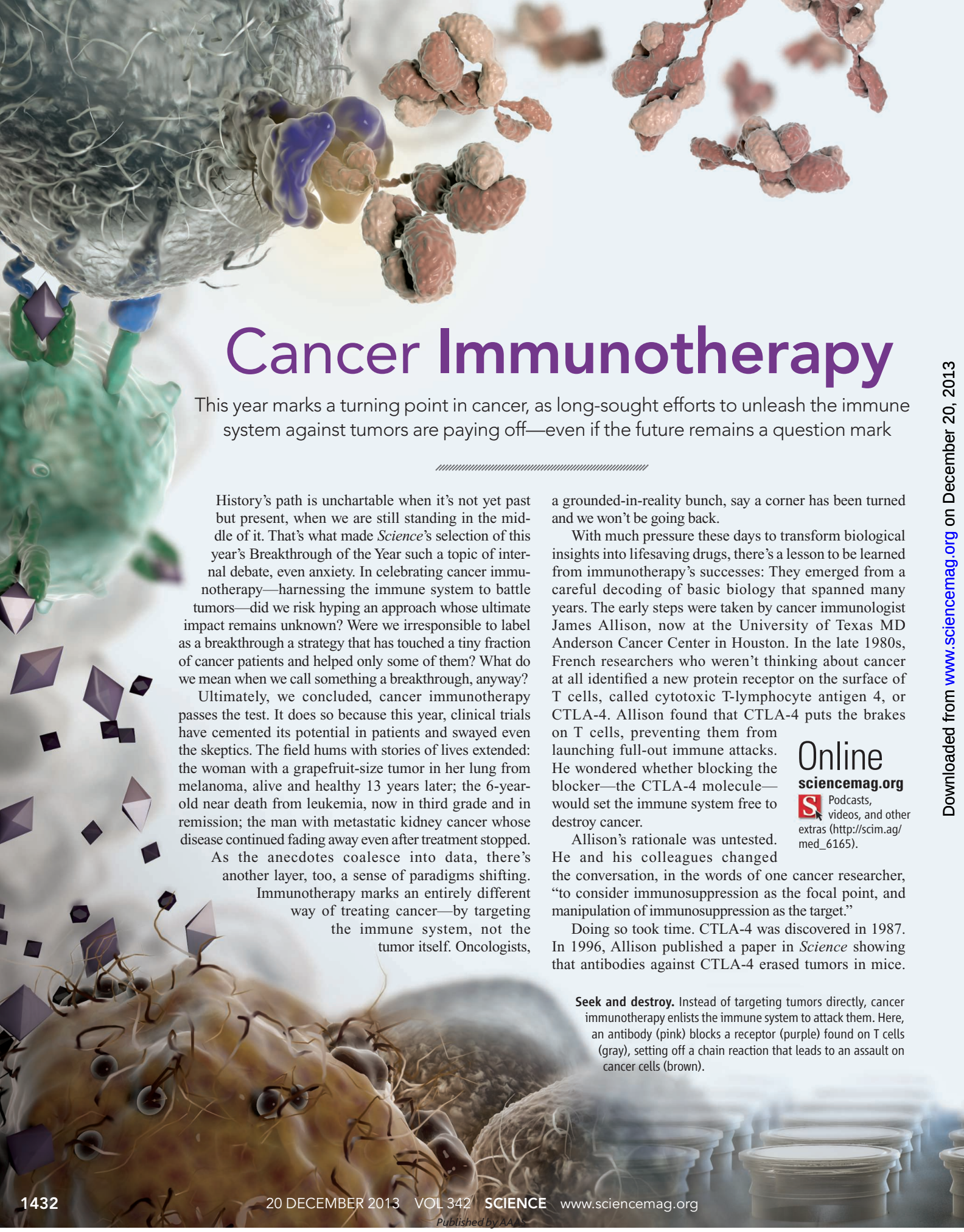
Dangerous imitator. Immune cells primed to identify invading viruses or bacteria sometimes attack the body's own cells when a foreign antigen (left) mimics a "self" antigen (right).

cells that are spurred to attack by hypocretin, a hormone that regulates wakefulness. Scientists knew that the neurons that produce hypocretin are missing in narcoleptic brains, but why they disappear is a mystery. The work reveals "the fingerprints of an immune attack," says neuroimmunologist Lawrence Steinman of Stanford University in Palo Alto, California, who was not involved in the research. It suggests that an autoimmune reaction, perhaps touched off by Pandemrix, could underlie the vaccine-linked cases. The researchers also speculate that flu itself might trigger other cases.

When the association between Pandemrix and narcolepsy showed up, the vaccine's

immunologist Elizabeth Mellins, and colleagues developed an immune system cell line that carries the HLA type associated with narcolepsy. They combined the cell line with short pieces of the hypocretin protein, then added T cells from patients and controls to the mix.

The team had access to four sets of identical twins in which one twin had narcolepsy but the other didn't. In each case, T cells from the affected twin reacted strongly to the hypocretin "epitopes" displayed by the HLA-bearing cells, but T cells from the healthy twin did not. The researchers found the same pattern when they compared T cells from 10 Irish children who had developed narcolepsy



Cancer Immunotherapy

This year marks a turning point in cancer, as long-sought efforts to unleash the immune system against tumors are paying off—even if the future remains a question mark

History's path is unchartable when it's not yet past but present, when we are still standing in the middle of it. That's what made *Science's* selection of this year's Breakthrough of the Year such a topic of internal debate, even anxiety. In celebrating cancer immunotherapy—harnessing the immune system to battle tumors—did we risk hyping an approach whose ultimate impact remains unknown? Were we irresponsible to label as a breakthrough a strategy that has touched a tiny fraction of cancer patients and helped only some of them? What do we mean when we call something a breakthrough, anyway?

Ultimately, we concluded, cancer immunotherapy passes the test. It does so because this year, clinical trials have cemented its potential in patients and swayed even the skeptics. The field hums with stories of lives extended: the woman with a grapefruit-size tumor in her lung from melanoma, alive and healthy 13 years later; the 6-year-old near death from leukemia, now in third grade and in remission; the man with metastatic kidney cancer whose disease continued fading away even after treatment stopped.

As the anecdotes coalesce into data, there's another layer, too, a sense of paradigms shifting.

Immunotherapy marks an entirely different way of treating cancer—by targeting the immune system, not the tumor itself. Oncologists,

a grounded-in-reality bunch, say a corner has been turned and we won't be going back.

With much pressure these days to transform biological insights into lifesaving drugs, there's a lesson to be learned from immunotherapy's successes: They emerged from a careful decoding of basic biology that spanned many years. The early steps were taken by cancer immunologist James Allison, now at the University of Texas MD Anderson Cancer Center in Houston. In the late 1980s, French researchers who weren't thinking about cancer at all identified a new protein receptor on the surface of T cells, called cytotoxic T-lymphocyte antigen 4, or CTLA-4. Allison found that CTLA-4 puts the brakes on T cells, preventing them from launching full-out immune attacks. He wondered whether blocking the blocker—the CTLA-4 molecule—would set the immune system free to destroy cancer.

Allison's rationale was untested. He and his colleagues changed the conversation, in the words of one cancer researcher, "to consider immunosuppression as the focal point, and manipulation of immunosuppression as the target."

Doing so took time. CTLA-4 was discovered in 1987. In 1996, Allison published a paper in *Science* showing that antibodies against CTLA-4 erased tumors in mice.

Online
sciencemag.org
Podcasts,
videos, and other
extras (http://scim.ag/med_6165).

Seek and destroy. Instead of targeting tumors directly, cancer immunotherapy enlists the immune system to attack them. Here, an antibody (pink) blocks a receptor (purple) found on T cells (gray), setting off a chain reaction that leads to an assault on cancer cells (brown).

Pharmaceutical companies shied away from cancer immunotherapy, wary of past flops but also of a strategy very unlike the standard zapping of a tumor. So the job of getting anti-CTLA-4 into people fell to a small biotechnology company, Medarex, in Princeton, New Jersey. In 1999, it acquired rights to the antibody, taking the leap from biology to drug.

Crucial results didn't come for another 11 years. In 2010, Bristol-Myers Squibb—which had bought Medarex for more than \$2 billion—reported that patients with metastatic melanoma lived an average of 10 months on the antibody, compared with 6 months without it. It was the first time any treatment had extended life in advanced melanoma in a randomized trial. Nearly a quarter of participants survived at least 2 years.

The numbers for another antibody are so far even better and the side effects milder. In the early 1990s, a biologist in Japan discovered a molecule expressed in dying T cells, which he called programmed death 1, or PD-1, and which he recognized as another brake on T cells. He wasn't thinking of cancer, but others did. One, oncologist Drew Pardoll at Johns Hopkins University, met with a leader of Medarex at a Baltimore coffee shop. He urged the company to test an anti-PD-1 antibody in people.

The first trial, with 39 patients and five different cancers, began in 2006. By 2008, doctors were jolted by what they saw: In five of the volunteers, all of them with refractory disease, tumors were shrinking. Survival in a few stretched beyond what was imagined possible.

Still, understanding what these therapies were doing inside the body was a challenge. Other cancer treatments either work or they don't, and the answer is nearly instantaneous. With both anti-CTLA-4 and anti-PD-1, physicians saw some tumors grow before vanishing months later. Some patients kept responding even after the antibody had been discontinued, suggesting their immune system had been fundamentally changed. Some, particularly those on anti-CTLA-4, developed unnerving side effects, inflammation of the colon, for example, or of the pituitary gland. All of these were the fine points of a new template, one whose vagaries physicians were just beginning to understand. The learning curve would be steep.

It was steep in another area of immunotherapy as well. For years, Steven Rosenberg at the National Cancer Institute had harvested T cells that had migrated into tumors, expanded them in the lab, and reinfused them into patients, saving some with dire prognoses. The technique worked only when doctors could access tumor tissue, though, limiting its application.

Then in 2010, Rosenberg published encouraging results

from so-called chimeric antigen receptor therapy, or CAR therapy—a personalized treatment that involves genetically modifying a patient's T cells to make them target tumor cells. One group, led by Carl June at the University of Pennsylvania, began reporting eye-catching responses to CAR therapy: patients with pounds of leukemia that melted away. At a meeting in New Orleans this month, June's team and another at Memorial Sloan-Kettering Cancer Center in New York reported that the T cell therapy in their studies put 45 of 75 adults and children with leukemia into complete remission, although some later relapsed. CAR therapy is now the focus of numerous clinical trials. Researchers hope that it, like the antibodies, can target an assortment of cancers.

Engineered T cells are still experimental, but the antibodies are slowly going mainstream. At least five major drug companies, their early hesitancy gone, are developing antibodies such as anti-PD-1. In 2011, the U.S. Food and Drug Administration approved Bristol-Myers Squibb's anti-CTLA-4 treatment, called ipilimumab, for metastatic melanoma. The cost is high: The company charges \$120,000 for a course of therapy. In 2012, Suzanne Topalian of Hopkins, Mario Sznol of Yale University, and their colleagues reported results for anti-PD-1 therapy in nearly 300 people, and they provided an update earlier this year. Tumors shrunk by about half or more in 31% of those with melanoma, 29% with kidney cancer, and 17% with lung cancer.

This year brought even more encouragement. Bristol-Myers Squibb reported this fall that of 1800 melanoma patients treated with ipilimumab, 22% were alive 3 years later. In June, researchers reported that combining ipilimumab and anti-PD-1 led to “deep and rapid tumor regression” in almost a third of melanoma patients. Drugs blocking the PD-1 pathway have not yet been proven to extend life, although survival rates so far have doctors optimistic that they do.

For physicians accustomed to losing every patient with advanced disease, the numbers bring a hope they couldn't have fathomed a few years ago. For those with metastatic cancer, the odds remain long. Today's immunotherapies don't help everyone, and researchers are largely clueless as to why more don't benefit. They are racing to identify biomarkers that might offer answers and experimenting with ways to make therapies more potent. It's likely that some cancers will not yield to immunotherapy for many years, if ever.

Even in the fluid state oncology now finds itself, this much is certain: One book has closed, and a new one has opened. How it will end is anyone's guess.

—JENNIFER COUZIN-FRANKEL

2013 Runners-Up

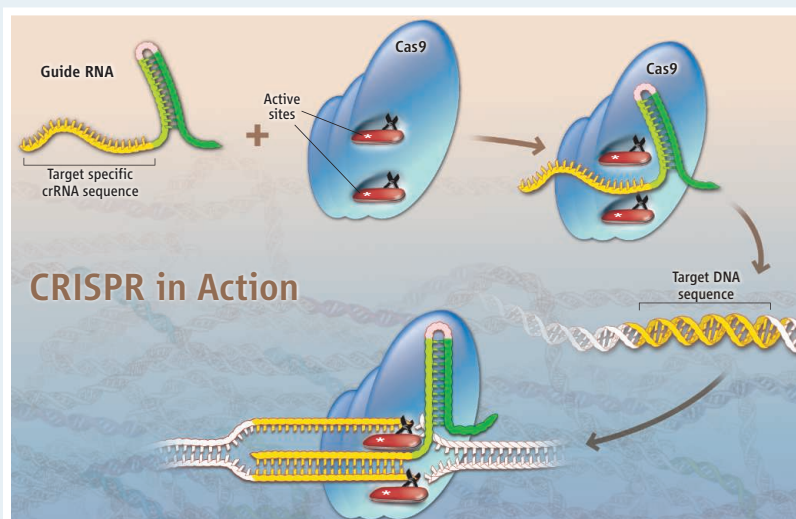
Many other results this year are also worth celebrating. Here, the best of the rest.

Genetic Microsurgery for the Masses

In the 1920s, the introduction of a microscope into the operating room touched off a revolution in surgical procedures. Suddenly, doctors could fix ears, blood vessels, and other parts of the human body with exhilarating precision and ease. Now, biologists operating on the genome are gaining similar abilities, thanks to a bacterial protein called Cas9. That protein, coupled with RNA designed to home in on specific DNA sequences, gives researchers the equivalent of a molecular surgery kit for routinely disabling, activating, or changing genes.

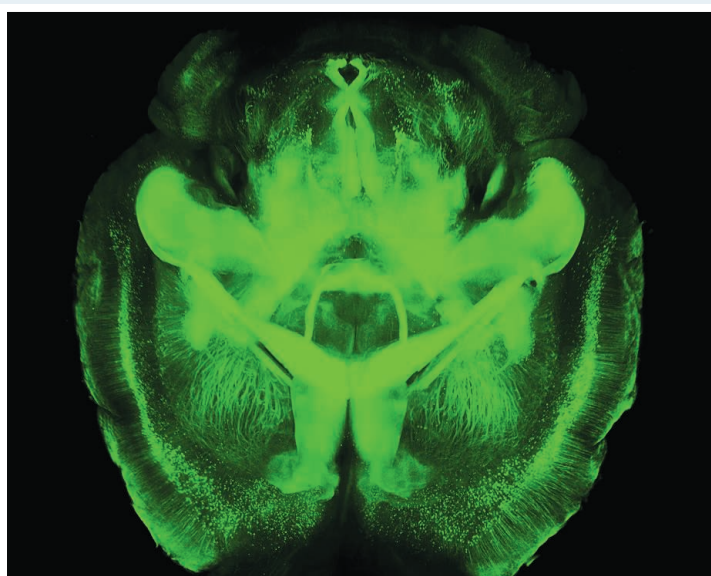
This technology, called CRISPR, has become red hot in the past year, with more than 50 publications in 10 months. One CRISPR “how-to” website now attracts about 900 visitors each day. Since January, more than a dozen teams have manipulated specific genes in mice, rats, bacteria, yeast, zebrafish, nematodes, fruit flies, plants, and human cells, paving the way for understanding how these genes function and possibly harnessing them to improve health. One team even reported using the approach to disable HIV hiding in T cells. CRISPR also has the potential of modifying multiple genes simultaneously, and it simplifies the job of making mouse models of disease.

Such genetic microsurgery was a dream a decade ago. Researchers could not readily control where an added gene would insert itself into a genome or which DNA would be deleted in so-called knock-out experiments. That changed about 10 years ago, when labs began



Molecular scalpel. To home in on the right DNA, the Cas9 protein links up with guide RNA that has a target-specific sequence. Once attached, Cas9 has two active sites that cut the DNA in the right place.

studying proteins called zinc finger nucleases. Their composition dictated which DNA sequence was cut, allowing researchers to cut DNA at will. Zinc fingers proved difficult to make. Then, just as that technology was beginning to take hold, another approach, based on a plant pathogen, provided an easier alternative. The rush to harness



CLARITY Makes It Perfectly Clear

A new window on the brain opened this year, promising to fundamentally change the way labs study the intricate organ. Called CLARITY, it turns brain tissue as transparent as glass by removing the fatty, light-scattering lipid molecules that form cellular membranes. CLARITY replaces the lipids with molecules of a clear gel but leaves all neurons, other brain cells, and their organelles intact, putting the intricacies of the brain on display.

Previous attempts to create see-through brains rendered them too fragile to work with, but

Crystal clear. A new method of making tissue transparent will help neuroscientists explore the postmortem brain in 3D.

that system, called TALENs (for transcription activator-like effector nucleases), prompted *Science* to recognize genome editing as one of the major achievements of 2012.

CRISPR, which stands for clustered regularly interspaced short palindromic repeats, takes genome editing to the next level. The name comes from repetitive stretches of DNA that bacteria have evolved as part of an adaptive immune system against viruses called bacteriophages. To fight these viruses, bacteria link the protein Cas9 to RNA that matches the virus's genome. The complex cuts the viral DNA, disabling it. In 2012, researchers first used lab-made CRISPR complexes for genome editing in a test tube. Others immediately recognized CRISPR's potential. With TALENs or zinc finger nucleases, each new gene targeted requires a custom protein be built. CRISPR substitutes RNA—which is simpler to make than a piece of a protein—for the DNA-targeting section.

Researchers are eagerly tinkering with CRISPR technology; they are modifying the Cas9 protein so that it nicks instead of cuts the DNA. Biochemists are also working out the structures of the Cas9 complexes. And a few labs are exploring whether other Cas proteins might work better than Cas9.

To take CRISPR from basic research to medicine—harnessing it to fix a broken gene or disable a bad one, for example—researchers must show that each CRISPR construct hits only its target and no others. For now, CRISPR RNA will sometimes latch on to DNA that doesn't exactly match up. But CRISPR researchers are already finding ways to sharpen their targeting.

Both CRISPR and TALENs were unanticipated outcomes of basic research unrelated to genome editing. So CRISPR could easily be displaced by an even slicker genome-editing tool. But for now, with companies forming and new studies coming out practically every week, the CRISPR craze is in full swing.

CLARITY leaves tissue sturdy enough for scientists to infiltrate it repeatedly with labels for specific cell types, neurotransmitters, or proteins; wash them out; and image the brain again with different labels. Researchers say the advance could speed up by 100-fold tasks such as counting all the neurons in a given brain region and could make traditional methods of imaging postmortem brain tissue irrelevant. At present, however, the technique is limited to small amounts of tissue: Just clarifying a 4-mm-diameter mouse brain takes about 9 days.

FOSSIL OF THE YEAR

Dmanisi Skull Gives New Face To Early Human Ancestors

Researchers unveiled the most complete skull of an early human ancestor this past November—and proved once again that a single fossil can transform our picture of human ancestors. The stunning 1.8-million-year-old remains of a mature male had a remarkably small brain and a large, jutting jaw. That's just the opposite of what researchers expected to find for members of our

genus *Homo* at this time. Four skulls of our direct ancestor, *Homo erectus*, found in the same sliver of time and place—at Dmanisi in Georgia—had bigger brains and less conspicuous mugs. As a result, the latest Dmanisi skull, which even includes the fragile midface bones, has given early *Homo* a new visage.

If it, too, is a member of *H. erectus*, as the researchers think, that species had more diversity in brain size and facial traits than previously believed. New dating of the skull



and its four companions also suggests that *H. erectus* left Africa soon after it appeared there 1.9 million years ago.

But some paleoanthropologists suggest the skull could belong to *Homo habilis*, a more distant earlier human ancestor that lived in Africa about 2.3 million to 1.4 million years ago. Or it could belong to a new species. Regardless of the skull's precise identity, its remarkable preservation will make it an icon for the face of early *Homo* for decades, if not centuries, to come.

VERTEBRATE OF THE YEAR

The Rat That Ages Beautifully

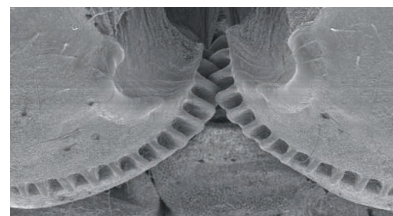
They will never win a beauty contest, but naked mole rats (*Heterocephalus glaber*) may hold a lesson or two for humans. Two studies this year, for instance, found clues to why these rodents can live 30 years, cancer-free. One secret may be a ribosome that excels at producing error-free proteins; misformed proteins can clog up the body's systems and accelerate aging. Another could be a supersized version of a complex sugar that seems to protect against cancer. Naked mole rats don't break this compound down as fast as other animals, so it builds up in the spaces between cells and may keep the cells from clumping together and forming tumors.



INVERTEBRATE OF THE YEAR

Top-Gear Planthopper

Turns out humans weren't the first organism to gear up to gain a powerful mechanical advantage. In September, high-speed videos revealed that



immature *Issus coleoptratus* planthoppers are such great leapers because of toothy interacting gears on their rear legs. By meshing together, the gears cock and coordinate the legs prior to and during each explosive hop.

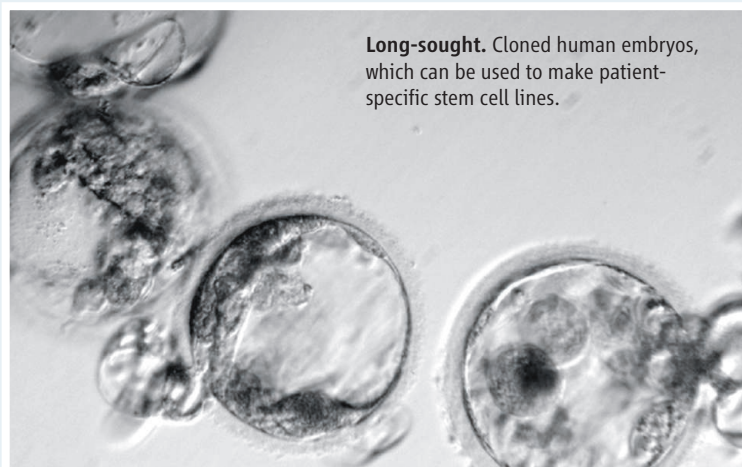
2013
Runners-Up

Human Cloning at Last

After more than a decade of failed attempts, it finally worked. This year, researchers announced that they had cloned human embryos and used them as a source of embryonic stem (ES) cells—a long-cherished goal. Able to develop into any tissue while providing a perfect genetic match to the cell that was cloned, the ES cells could prove a powerful tool for research and medicine. However, concerns about destroying embryos and the emergence of a cheaper, easier rival technique might keep human cloning for stem cells from becoming standard practice.

The cloning technique, called somatic cell nuclear transfer (SCNT), is the same one used to clone Dolly the sheep 17 years ago. Scientists remove the nucleus from an egg cell and then fuse the remaining cell material with a cell from the individual to be cloned. They then give the fused cell a signal to start dividing, and when things go right an embryo develops. Scientists have used SCNT to clone mice, pigs, dogs, and other animals, but human cells proved trickier to work with. Years of trying—and a high-profile fraud—yielded nothing more than a few poor-quality embryos, unable to produce ES cells.

At the Oregon National Primate Research Center in Beaverton, however, researchers finally cloned monkey embryos and derived ES cells from them in 2007. In the process, they discovered a number of tweaks that made SCNT more effective for primate cells, including human ones. The final recipe

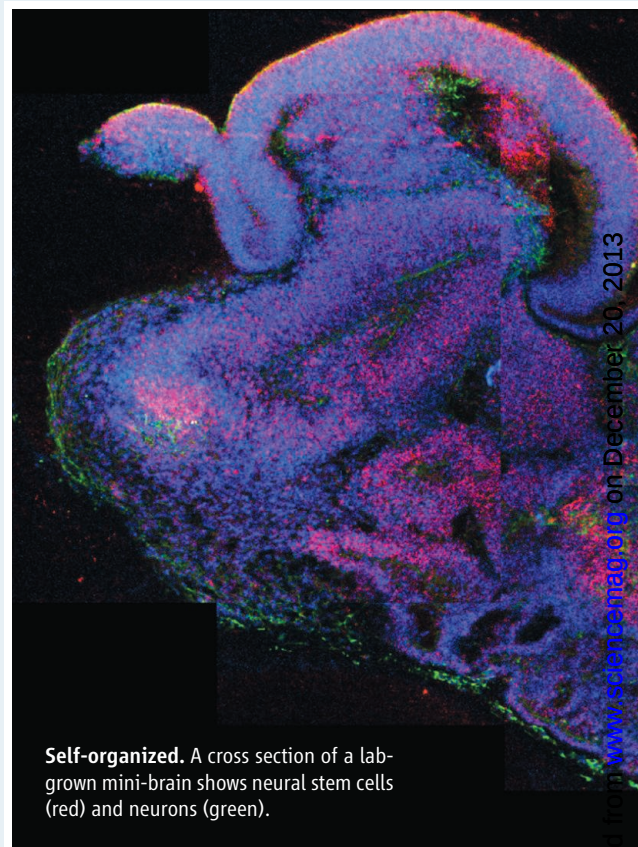


Long-sought. Cloned human embryos, which can be used to make patient-specific stem cell lines.

worked surprisingly well, yielding ES cells in about one in 10 tries. One key ingredient seems to be caffeine, which appears to help stabilize key molecules in the delicate human egg cells.

How important the technique will be in the long run is an open question. In the years since human cloning was first attempted, researchers found that they can make patient-specific stem cells by “reprogramming” adult cells into induced pluripotent stem (iPS) cells. That method, which scientists adapted to human cells in 2007, eliminated the need for human eggs and does not involve embryos, two aspects that make SCNT controversial and expensive. But some experiments have suggested that, at least in mice, ES cells from cloned embryos might be of better quality than iPS cells. Now, investigators will be able to compare the two types of human stem cells side by side.

The feat also raises concerns about cloned babies. But that seems unlikely for now. Despite hundreds of tries, the Oregon researchers say, none of their cloned monkey embryos have established a pregnancy in surrogate females.

Dishing Up
Mini-Organs

Self-organized. A cross section of a lab-grown mini-brain shows neural stem cells (red) and neurons (green).

Left alone in a lab dish, pluripotent stem cells run riot. They differentiate into a disorganized mass of tissues: beating heart cells, neurons, even hair and teeth. It's still a challenge to coax stem cells to grow into specific tissues, let alone into organized structures. This year, researchers did just that, in spectacular style, growing a variety of “organoids” in the lab: liver buds, mini-kidneys, and, most remarkably, rudimentary human brains.

The brains, grown by Austrian researchers, differ in important ways from the real thing. Because they have no blood supply, they stop growing once they reach the size of an apple seed. Cells at the core, starved of oxygen and other nutrients, die off. But the organoids mimic developing human brains to a surprising degree, developing eye tissue and layers that, under the microscope, resemble those in the brain of an early human fetus.

Inspired by previous work that had grown mini-guts from stem cells, the researchers began by encouraging human embryonic stem cells and

BREAKOUT OF THE YEAR

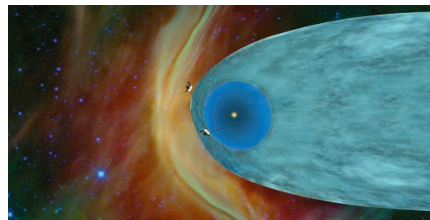
Voyager Is Really Out There, Somewhere

Thirty-six years out from Earth, its power dwindling and several instruments dead, the Voyager 1 spacecraft has left the invisible cocoon spun by the sun and entered interstellar space. So concluded Voyager team leaders and most space physicists in September. But it had taken them a year to realize Voyager had broken out of the heliosphere—the bubble inflated by the sun's wind of charged particles. That is a testament to just how weird the outer "edge" of the solar system proved to be.

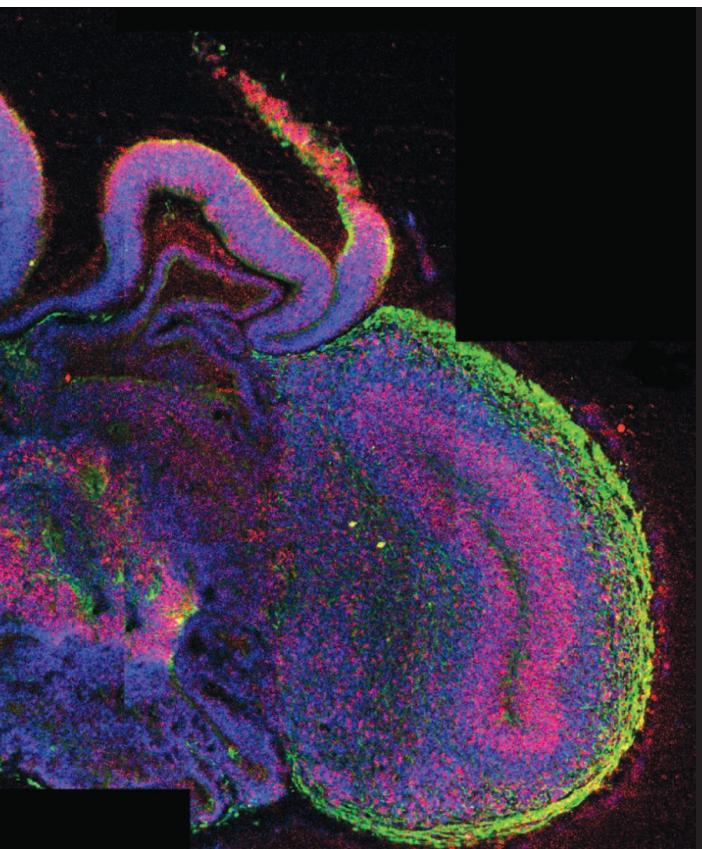
Team members had a checklist of indicators that would confirm that Voyager 1 had crossed into interstellar space. The density of plasma—the soup of low-energy charged and neutral particles pervading space—should jump, cosmic rays produced within the heliosphere should drop while those of interstellar space should increase sharply, and the direction of the magnetic field that pervades all space should switch. Voyager couldn't detect any change in plasma density, because its plasma instrument failed shortly after passing by Saturn. It did measure the expected changes in cosmic rays, in August 2012. But it never saw the magnetic field switch. So the official team line had Voyager in a "depletion region" still within the heliosphere.

There, Voyager would have remained if not for a little help from the sun. Twice it sent a solar blast out Voyager's way, setting off oscillations in the plasma that the spacecraft's plasma wave instrument could detect, giving team members a proxy for plasma density. Extrapolating back, they could see that the plasma density had shifted in August 2012, just when cosmic rays had switched. Team leaders concluded that Voyager 1 had actually entered interstellar space at that time, even though the magnetic field did not shift.

That interpretation will be tested as Voyager 2, a few years behind its sibling, brings its operating plasma instrument to bear on the same region.



Going, gone. Voyager 1 (*top*) has exited the heliosphere in this artist's conception, while Voyager 2 (*bottom*) is getting close.



induced pluripotent stem (iPS) cells to become neural stem cells. Then they suspended clumps of the cells in a gelatinous material called Matrigel and let them grow in a bioreactor, which rotates to help nutrients reach the cell clusters.

To the scientists' surprise, after a few weeks they saw darker pigmented cells that seemed to resemble early eye development. On closer inspection, they found evidence that the organoids had developed layers identifiable as forebrain, midbrain, and hindbrain, all typical of fetal brains. They also saw evidence for an outer subventricular zone, which is present in human brains but not mouse ones.

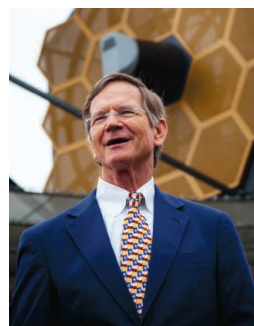
The mini-brains have already yielded insights into microcephaly, a condition in which the brain doesn't grow to its full size. When the team started with iPS cells derived from a microcephaly patient, the resulting organoids were smaller than normal because stem cells stopped dividing too soon. With further development, researchers hope to use the mini-brain technique to investigate other brain diseases.

CREDITS (TOP TO BOTTOM): NASA/JPL-CALTECH; NASA

POLITICO OF THE YEAR

Chairman Smith Versus the Scientists

U.S. Representative Lamar Smith (R-TX) likes to recall how a "D" in a freshman physics class at Yale University taught by a former presidential science adviser, D. Allan Bromley, caused him to switch his major to American studies



—and started him on the road to a career in politics. Now, the tables have turned: This year, Smith gave a failing grade to the National Science Foundation (NSF) as part of a controversial attempt to reshape U.S. science policy that has scientists talking.

As the new chair of the House of Representatives science committee, Smith has drafted legislation that would alter how NSF manages peer review. He says the proposed changes would make the system more transparent and ensure that tax dollars are being spent

wisely. But science leaders view the bill as a threat to a system that has fueled 60 years of innovation—and that other nations are trying to copy.

In a bid to preempt the draft legislation, this month NSF announced plans to sharpen its descriptions of funded grants to emphasize their relevance to important societal goals. Will it be enough?

2013
Runners-Up

Cosmic Particle Accelerators Identified



For decades, physicists thought they knew where many of the immensely energetic protons and atomic nuclei that whiz in from space as cosmic rays get their start: in the wreckage of exploded stars, or supernovas. Now they're sure. This year, researchers with NASA's orbiting Fermi Gamma-ray Space Telescope produced the first direct evidence of such particles revving up in cloudlike supernova remnants within our galaxy.

When a star explodes, material ejected from it crashes into a tenuous sea of gas between the stars. That interstellar medium is so thin that few particles collide directly. However, particles from the supernova can rebound off magnetic fields in space, twisting them up to form a lingering collisionless shock that slingshots other particles to higher energies. In the late 1970s, theorists realized that as protons and nuclei circulate repeatedly through such a shock, they may accelerate to colossal energies—hundreds of times higher than particle accelerators have reached.

But tracing cosmic rays to supernova remnants

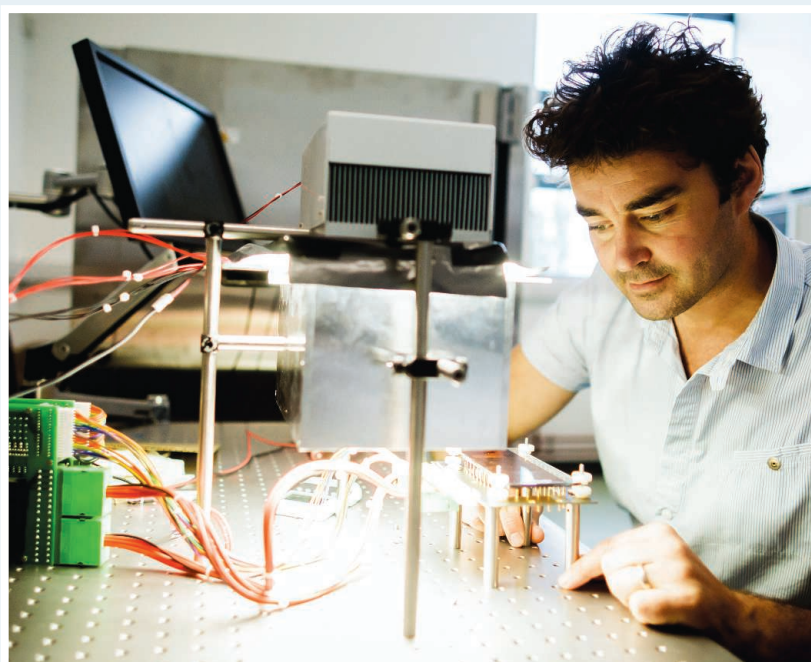
Boom! Supernova remnants such as the Jellyfish Nebula can boost particles to enormous energies.

Newcomer Juices Up the Race to Harness Sunlight

A rising star lit up the world of solar power research this year. Cheap, easy-to-make crystals called perovskites proved capable of converting more than 15% of the energy in sunlight to electricity. That's up from 3.8% just 4 years ago, and it's already better than a couple of other solar cell technologies that researchers have been working on for decades.

Perovskite solar cells still lag behind the silicon panels that dot rooftops worldwide. Those typically achieve about 20% efficiency, and the best performers in research labs reach 25%. But silicon solar cells and other high-performance solar materials rely on semiconductors that must be grown at high temperatures in expensive fabrication facilities. Not so with perovskites. The versions used for solar cells thus far are made simply by mixing inexpensive precursor compounds in solution and then drying them on a surface. Surprisingly, this procedure produces perovskites with such high crystalline quality that two groups reported using them to make lasers, an

Sunny side up. Solar cell materials known as perovskites burst on the scene promising cheap, high-efficiency solar power.



CREDITS (TOP TO BOTTOM): NASA/DOE/FERMI LAT COLLABORATION; TOM BASH AND JOHN FOX/ADAM BLOCK/NOAO/AURA/NSF; JPL-CALTECH/UCLA; PIRANHA PHOTOGRAPHY/OXFORD PHOTO

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hasn't been easy. Because they're electrically charged, protons and nuclei swirl in interstellar magnetic fields. So by the time they reach Earth, cosmic rays do not point back to their birthplaces. The Fermi team had to find another way to show that supernova remnants accelerate the particles.

If protons are accelerated in a supernova remnant, then a few proton-proton collisions should still occur. Such collisions produce fleeting particles called pi-zero mesons, each of which quickly decays into a pair of high-energy photons. Those pi-zero decays should then produce a telltale hump in the energy spectrum of photons from a supernova remnant, when it's plotted in a particular way. After 5 years of collecting data, Fermi researchers spotted that signature of proton acceleration in two supernova remnants. They had to tease it out of the overwhelming glare of photons that ricochet off high-energy electrons, which do not have such a spectral bump. Others had looked for the signature, but Fermi is the first experiment to see it clearly.

Astrophysicists still don't know many details of how the particles and magnetic fields interact, and they suspect that the highest energy cosmic rays originate from other sources outside our galaxy. Still, there's now no doubting that supernova remnants do indeed spew cosmic rays.

application for which near-perfect crystals are de rigueur.

Crystalline quality is central to a solar cell's ability to produce power. When sunlight strikes a cell, it energizes electric charges. This propels them through the material to the electrodes, where they are collected and sent through wires as an electric current. Defects in semiconductor crystals act as speed traps. Perovskites offer a cheap route to fewer traps.

But perhaps the best news about perovskite solar cells is that it may be possible to integrate them with conventional silicon solar cells, layering the newbies right on top of silicon panels. Perovskites excel at snagging the higher energy photons in sunlight—the blues and greens—while silicon does better at grabbing the lower energy red and infrared photons. So putting the two together in a hybrid could achieve an efficiency of as much as 30%. Solar researchers around the globe are racing to marry the two materials.

They're also coping with potential problems. Solar cell perovskites are fragile and readily break down when exposed to water or air. The current varieties also contain lead, an environmental toxin. To make a viable technology, researchers will have to find ways to encapsulate the materials, and find safer replacements. If the rapid progress so far can be sustained, perovskites' star has only begun to rise.

BREAKUP OF THE YEAR

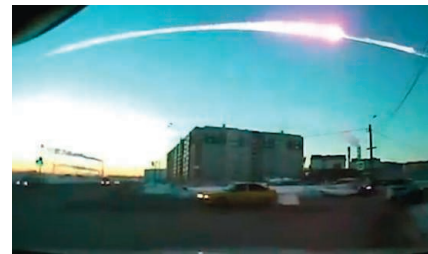
Siberian Meteor Blast Delivers a Warning Shot

February's window-shattering explosion over Chelyabinsk, Russia, terrified thousands and caused hundreds of injuries, mostly minor. It also provided a windfall for scientists and a public relations bonanza for asteroid hunters.

For scientists, the midair self-destruction of a 19-meter-diameter rock provided a key benchmark for re-evaluating the threat from such relatively small visitors from the asteroid belt. Researchers had plenty of observations to work with. Data came from 400 public and private video cameras, seismographs, ground-based sensors that record ultra-low-frequency sound, and satellites intended to catch clandestine nuclear tests.

The Chelyabinsk blast had an energy equivalent of about 500 kilotons of TNT, researchers estimate, or about 23 times the energy of the nuclear bomb dropped on Nagasaki. Initially, that output appeared to make the airburst a rarity, far larger than expected by astronomers who use telescopic surveys to gauge asteroid hazards. But after researchers compared the sound and brightness of Chelyabinsk with other meteor airbursts over a 20-year period, they found that big airbursts are at least three times—and perhaps 10 times—more frequent than astronomers thought.

The incident energized those eager to find other small asteroids, whether heading for Earth or passing nearby. NASA is cranking up its search effort because it wants to find an asteroid smaller than 10 meters for an astronaut rendezvous. The nonprofit B612 Foundation wants to fly a spacecraft-borne telescope to help with that mission and search for hazardous asteroids, if someone will provide the funding.



RULING OF THE YEAR

U.S. High Court Bars Human Gene Patents

In June, the U.S. Supreme Court reversed three decades of policy—and rattled the biotech world—when it ruled that human genes cannot be patented. The case, *Association for Molecular Pathology v. Myriad Genetics*, pitted a Utah company that owns patents on the human breast cancer genes *BRCA1* and *BRCA2* against doctors and researchers who argued that the patents were

invalid and stifling research. The court agreed unanimously, finding that human genes are a "product of nature" and so not patentable.

It will take years for the full consequences of this revolution in legal thinking to become clear. But the decision is already making waves. It

spurred several companies to offer breast cancer diagnostic tests that competed with Myriad's—sparking a contentious new legal battle. And in October, federal Judge Susan Illston in San Francisco cited the Supreme Court's reasoning in striking down another DNA patent, involving a Down syndrome test developed by Sequenom of San Diego, California. Sequenom plans to appeal, and it is likely that many companies will be asking judges to clarify exactly how the law defines a product of nature.



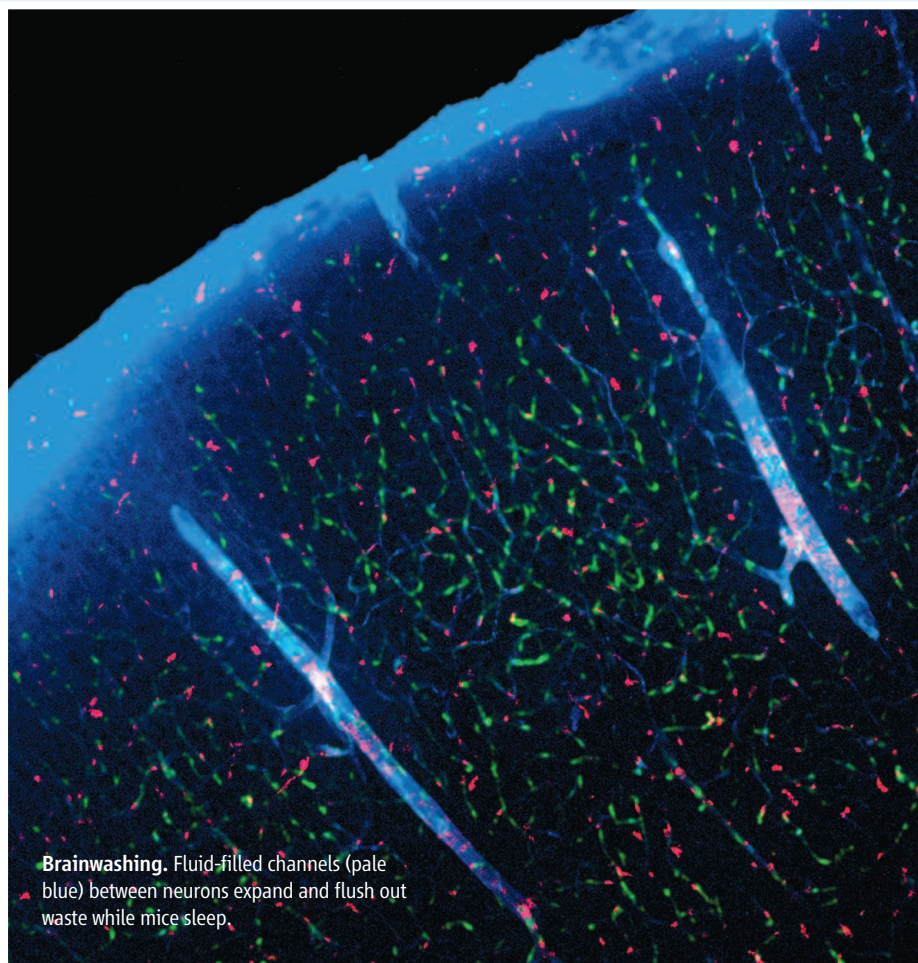
2013
Runners-Up

To Sleep, Perchance to Clean

Why do we sleep? Questions of biology don't get much more fundamental than that. This year, neuroscientists took what looks like a major stride toward an answer.

Most researchers agree that sleep serves many purposes, such as bolstering the immune system and consolidating memories, but they have long sought a "core" function common to species that sleep. By tracking colored dye through the brains of sleeping mice, scientists got what they think is a direct view of sleep's basic purpose: cleaning the brain. When mice slumber, they found, a network of transport channels through the brain expands by 60%, increasing the flow of cerebral spinal fluid. The surge of fluid clears away metabolic waste products such as β amyloid proteins, which can plaster neurons with plaques and are associated with Alzheimer's disease.

Until this discovery, researchers thought the brain's only way to dispose of cellular trash was to break it down and recycle it inside cells. If future research finds that many other species undergo this cerebral housekeeping, it would suggest that cleaning is indeed a core function of sleep. The new findings also suggest that sleep deprivation may play a role in the development of neurological diseases. But with a causal role far from certain, it's too early for anyone to stay awake worrying.



Brainwashing. Fluid-filled channels (pale blue) between neurons expand and flush out waste while mice sleep.



Your Microbes, Your Health

One hundred trillion cells bearing 3 million different genes—that's the roster of microbes that live inside your body. They're not just passengers; if animal studies hold up, these unseen multitudes profoundly affect the body's response to the environment, illness, and medical treatment. This year, researchers started pinpointing specific ways in which the microbiome promotes health and disease.

Gut sense. Microbes may be key players in malnutrition and other aspects of health.

- In 2008, nearly 300,000 infants in China got kidney stones from milk formula tainted with melamine, a plastics additive that was used illegally to bulk up the formula's apparent protein content. This year, scientists found that a bacterium may be to blame. A study showed that rats exposed to melamine developed fewer kidney stones when they were given antibiotics. The reason: The treated rats lacked *Klebsiella*, which con-

Breakdowns of the Year

In May, for the first time in recorded history, atmospheric concentrations of carbon dioxide **rose above 400 parts per million**, dramatizing the failure of governments to limit greenhouse gas emissions. • The same month, a **second reaction wheel failed** on the Kepler planet-hunting spacecraft, dooming its ability to point accurately and collect precise data.

• In October, congressional gridlock on spending issues forced **the U.S. government to partially shut down** for 16 days, paralyzing science funding agencies, disrupting research projects, and canceling many Antarctic field studies. • More than one-half of 304 free open-access journals **accepted a bogus paper** submitted by *Science* journalist John Bohannon, who sparked fierce debate over the quality of peer review when he reported his sting. • By year's end, disgraced Dutch social psychologist Diederik Stapel had **retracted at least 54 papers** based on made-up data, but was giving a TED talk about his misconduct.



Genomes of the Year

Notable sequences of 2013: The **oldest human mitochondrial DNA**, which comes from a 400,000-year-old Neandertal ancestor found in Spain but mysteriously resembles that of a different extinct human • The oldest organismal genome, from a **700,000-year-old frozen horse hoof** • Other complete genomes came from the **comb jelly**, changing views of the animal tree of life • **Minke whale**, revealing how marine mammals cope with deep dives • **Amborella**, sister to all flowering plants, explaining the early days of angiosperms • Tiger, lion, and snow leopard, capturing the **genomic essence of big cats** • The scorpion

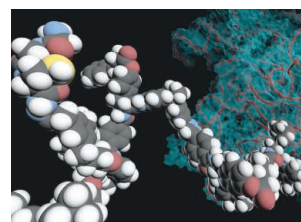


Mesobuthus martensii (at right), which has 10,000 more genes than humans do • **Norway spruce** (*Picea abies*), **white spruce** (*Picea glauca*), and, soon, **loblolly pine** (*Pinus taeda*), each with genomes about seven times the size of a human's—a sequencing tour de force • The invaluable **HeLa cancer research cell line**, requiring permission from the family of Henrietta Lacks • **King cobra** and **Burmese python**, telling an evolutionary tale of extreme adaptations • **Four bats** which, when compared to dolphins, highlight a common core of echolocation genes • **Pigeon**, revealing the gene for crests • **Irish famine potato blight**, showing that this historic strain is extinct.

ALSO NOTED

Ribosome Robot

In January, one of the world's first nanofactories made its debut. Researchers built a molecular machine that mimics the cell's protein-building factory, the ribosome, but is one-tenth the size. This ribosome robot includes a molecu-



lar axle track with a ring around it. When heated, the ring's sulfur-containing amino acid sequentially grabs each of three amino acids from the track to build a short peptide chain. The machine has been hailed as ingenious, but it won't replace ribosomes anytime soon. It takes days to do what the real ribosome can do in tenths of a second.

verts melamine to a form that collects in the kidney. About 1% of infants carry *Klebsiella*—about the same percentage of infants on milk formula who got sick, suggesting this microbe may play a role in human toxicity as well.

• In Malawi, researchers studied unusual cases in which one twin developed a malnutrition syndrome called Kwashiorkor but the other did not. They sampled the children's microbes for 3 years, tracking how the bacterial populations changed before, during, and after treatment with a nutritional supplement. They also implanted fecal material from each twin into the guts of germ-free mice and then monitored the animals over several weeks. Mice that received bacteria from the Kwashiorkor children developed Kwashiorkor-like symptoms; other mice did not. The researchers discovered that the malnourished children's microbial portfolio had not matured properly. As a result, they suggest, the children were less able to process amino acids containing sulfur and thus were more prone to malnutrition.

• This year, researchers traced several links between gut microbes and cancer. Three anticancer therapies proved to need gut bacteria to be effective; the bacteria help prime the immune system to respond to drug treatment. A mouse study showed that a liver cancer often associated with obesity can arise because of a DNA-damaging bacterial byproduct that builds up in obese mice. Finally, new results confirmed earlier hints that a gut bacterium called *Fusobacterium* plays a role in stimulating colorectal tumors.

• In a study of obese mice, the animals lost weight and had better insulin control—even on a high-fat diet—when researchers boosted the amount of the mucus-eating gut bacterium *Akkermansia muciniphila* in their guts. Obese mice, as well as obese people and people with type 2 diabetes, typically have reduced numbers of these bacteria. The same bacterium also seems to play a role in the weight loss that accompanies gastric bypass surgery.

The year also saw more tantalizing hints of microbial influences on immune system function. The autoimmune disease rheumatoid arthritis, for example, may be associated with a bacterium called *Prevotella copri*. In mice, increases in the bacterium *Lactobacillus johnsonii* in the gut account for much of the protection against allergies and asthma provided by exposure to indoor/outdoor dogs and, to a lesser extent, cats.

The studies make it increasingly clear that personalized medicine will need to take our microbial guests into account to be most effective.

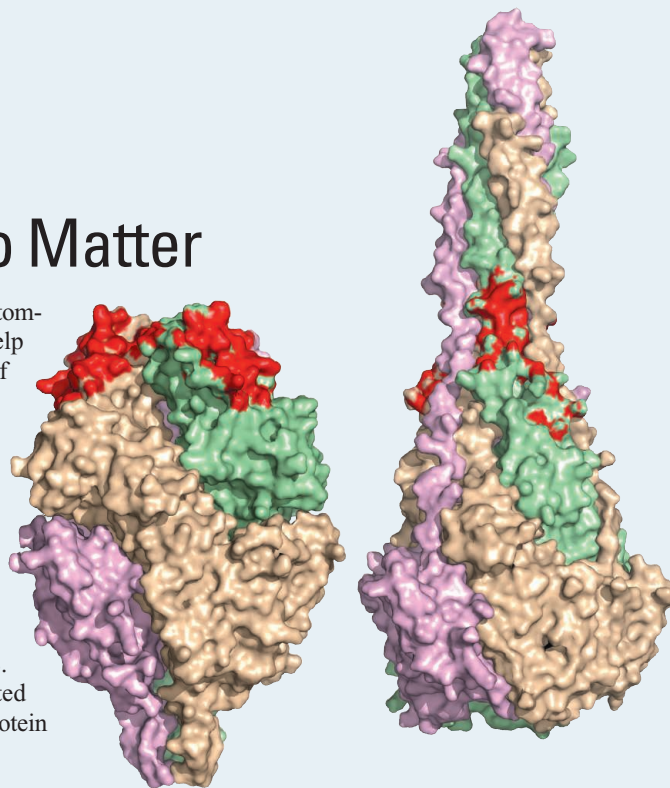
2013
Runners-Up

In Vaccine Design, Looks Do Matter

For decades, researchers have hoped that structural biology—the near-atom-level study of the molecules that make up living organisms—would help them design better vaccines. This year, they finally found convincing proof that the approach can deliver big-time payoffs.

Respiratory syncytial virus (RSV) hospitalizes millions of infants each year with pneumonia and other lung diseases, and it has defied many a vaccine developer. For children at high risk of developing severe RSV disease—which worldwide kills 160,000 kids each year—a monoclonal antibody on the market, palivizumab, can cut the risk of hospitalization in half. But palivizumab must be used repeatedly and costs nearly \$1000 a dose, placing it far out of reach of many.

Antibodies that have 10- to 100-fold more potency than palivizumab recently have been isolated, and in May, a research team at the U.S. National Institute of Allergy and Infectious Diseases (NIAID) reported that it had crystallized one of them in action. The antibody binds to a protein



CREDIT: JASON MCLELLAN/NIAID VACCINE RESEARCH CENTER

How We Did in 2013 and ...

Each year in this section, the news team hazards a few predictions about things to come in the months ahead. First, though, let's see how our forecasts for 2013 fared

**1. One Cell at a Time**

Though not yet routine and nowhere near the clinic, single-cell DNA sequencing is yielding important discoveries such as unexpected variation in supposedly similar cells, including brain cells.

**2. Planck Maps the Cosmic Microwave Background**

As expected, Europe's Planck spacecraft mapped the cosmic microwave background with unprecedented accuracy. But the Planck team's analysis merely confirmed the standard theory of what the universe is made of and how it evolved. Planck researchers will get one more shot at a major discovery—see "Cosmic history, with a twist" on page 1443.

**3. Connectomes**

The \$40 million Human Connectome Project released the first raw data in its 5-year quest to learn how millimeter-scale variations in the connections between human brain regions might account for individual differences in cognition and behavior. Other researchers this year mapped a speck of mouse brain synapse by synapse.

**4. Piercing a Frigid Underworld**

In January, Russian drillers retrieved a core of ice frozen from Lake Vostok, a pocket of water buried nearly 4000 meters below the Antarctic ice sheet. Scientists were eager to see what sort of microbial life the lake might harbor after millions of years of isolation. But the cells they spotted may have ridden down to Vostok on contaminated drills; more sampling could be necessary.

**5. Cancer Immunotherapy**

It's our Breakthrough of the Year—enough said.

**6. Plant Power**

This year, seed companies introduced drought-tolerant crops and scientists pinpointed new regulators of plant growth and development. Two teams also homed in on a gene that can fend off Ug99, or wheat stem rust, a global threat. Scientists are hard at work developing new varieties adapted to anticipated future conditions. But two expected payoffs from basic research—commercial algae-based fuels and forecasts of how plants will fare under climate change—have not yet materialized.

Packed punch. The RSV F protein best displays the red area needed to trigger potent antibodies in its coiled state (*left*) before fusing with a cell.

on RSV's surface, dubbed F, that the virus employs to fuse with cells during the infection process. Using x-ray diffraction techniques to study the crystal structure of the bound antibody, the researchers mapped precisely where it attaches to the F protein. Other potent, novel antibodies allowed the team to analyze the vulnerable site on the F protein in even finer detail.

In November, the same NIAID group described the next step: using the findings from their structural analyses to design an RSV F protein that could serve as an immunogen (the main ingredient of a vaccine). The F protein is like a jack-in-the-box: coiled up before it fuses with a cell and "sprung" afterward. The coiled, prefusion state best displays the vulnerable site. To teach an immune system to make potent antibodies, the researchers reasoned, a vaccine would have to contain the F protein locked into a prefusion configuration that features a full-monty view of the vulnerable site.

The investigators engineered just such a prefusion F protein and injected it into animals. Their strategy was vindicated: The protein

stimulated production of highly potent antibodies. Overnight, it became a leading candidate in the race to develop an RSV vaccine. It has yet to go into humans, but the NIAID researchers hope to have a product ready for testing in 18 months.

Three other studies published this fall exploited similar strategies to design a vaccine for another intractable infection, HIV. By mapping antibody-binding sites on an HIV surface protein, researchers identified features that may be essential to a successful vaccine. They designed a novel, "near native" version of the surface protein that displayed those features to best advantage. The investigators still have yet to prove that their putative immunogen can stimulate antibodies capable of tripping up the myriad variants of HIV in circulation, but they hope to follow in the footsteps of the RSV colleagues, who tested many versions of their artificial proteins in animal experiments to find the best one.

Now that structural biology has proven its value to vaccine design, many researchers hope the groundbreaking work will point the way toward vaccines for hepatitis C, dengue, West Nile, and other viruses that have perfected the art of dodging immune attack. Similar up-close-and-personal analyses of viruses might also reveal why so many viral diseases have eluded effective treatment for so long.

CREDITS (LEFT TO RIGHT): C. BICKEL/SCIENCE, GLOBALPI/ISTOCKPHOTO

... Areas to Watch in 2014

Space ghosts. This year, physicists finally detected elusive particles called neutrinos coming from beyond our solar system. Now, the question is whether they will prove to be a useful tool for probing the uni-



verse. The observations from IceCube, a massive array of particle detectors sunk into 1 cubic kilometer of ice at the South Pole, completed a 40-year quest for cosmic neutrinos. But IceCube's main aim is to pinpoint their sources in the sky and usher in the field of neutrino astronomy, and it remains to be seen if it can collect enough of the ghostly particles for that.

The IceCube team's first earnest attempt to map the neutrino sky should come within months, so scientists may soon know whether the array is big enough to serve as a neutrino telescope or whether something more is needed.

Clinical genomes. In 2014, more and more researchers—and even some doctors—will be requesting a patient's entire genome sequence or a subset of it, the protein-coding DNA known as the exome. The results could help diagnose rare diseases and identify cancer treatments. Several studies will explore whether sequencing should become part of newborn screening and even guide the medical care of healthy people. In perhaps the most ambitious clinical sequencing project yet, the United Kingdom will embark on a 4-year plan to sequence the genomes of 100,000 patients, most of them with cancer and rare diseases, to guide their treatments. Looming over these efforts is a debate over whether patients should be notified when the sequencing reveals "incidental" results—medically important findings unrelated to their illness.

Cosmic history, with a twist. The afterglow of the big bang—the so-called cosmic microwave background (CMB) radiation—may soon yield another revelation about how the universe began. That primordial radiation could contain traces of gravity waves that rippled through the universe in the first sliver of a second. Those traces would take the form of swirling patterns in the polarization of the microwaves as the CMB is mapped across the sky. The swirls, called B modes, could yield clues to how the universe underwent a faster-than-light growth spurt known as inflation. Researchers with the European Space Agency's spacecraft Planck, which took data from August 2009 to October 2013, should release a polarization map of the whole sky within months. But some researchers say Planck may not have the resolution to spot the swirls and may get scooped by ground-based efforts.

Bye-bye, chimps? One way or another, chimpanzees may be leaving U.S. research labs. In June, the National Institutes of Health announced plans to retire all but 50 of its 360 research chimps and phase out much of the chimp research it supports. The U.S. Fish and Wildlife Service, meanwhile, has recommended that captive chimps be listed as endangered, which would limit any research that isn't in their best interest. And just this month, an animal rights group known as the Nonhuman Rights Project launched a legal effort to have chimpanzees declared legal persons and freed from captivity. Its first three lawsuits, filed in three New York courts, were dismissed, but it plans to appeal (<http://scim.ag/NHRPchimps>). Scientists who study the animals may soon have to move on to other creatures—or abandon their research entirely.



LETTERS

edited by Jennifer Sills

Sea-Level Rise by 2100

IN HIS NEWS AND ANALYSIS PIECE REPORTING ON THE NEWLY RELEASED FIFTH ASSESSMENT report (AR5) by Working Group I (WGI) of the Intergovernmental Panel on Climate Change (IPCC) ("A Stronger IPCC Report," 4 October, p. 23), R. A. Kerr highlights three fundamental conclusions about climate change that were assessed with equal or greater confidence than in previous IPCC reports. He also points to three "contentious points" on which he states that the AR5 "took a moderate line." Kerr includes sea-level projections among these points, and reports "a rise of 40 to 60 centimeters by late in the century and a worst case of 1 meter by 2100, [which is] higher than in 2007 but far below the meter or two of sea-level rise that some expect."

As the authors of the IPCC WGI AR5 chapter on "Sea-Level Change," we wish to clarify that for the highest emission scenario considered (RCP8.5), the AR5 reported a "likely" range of 0.45 to 0.82 m for sea-level projections for the late 21st century (average over 2081 to 2100) and of 0.52 to 0.98 m by 2100. The difference in sea level between these two periods is large because in 2081 to 2100, the "likely" rate of rise is 8 to 16 mm per year, which is up to about 10 times the average rate of rise during the 20th century.

In the calibrated uncertainty language of the IPCC, this assessed likelihood means that there is roughly a one-third probability that sea-level rise by 2100 may lie outside the "likely" range. That is, the AR5 did not exclude the possibility of higher sea levels. However, we concluded that sea levels substantially higher than the "likely" range would only occur in the 21st century if the sections of the Antarctic ice sheet that have bases below sea level were to collapse. We determined with medium confidence that "this additional contribution would not exceed several 10ths of a meter of sea-level rise during the 21st century." We could not define this possible contribution more precisely because "there is currently insufficient evidence to evaluate the probability of specific levels above the assessed 'likely' range."

The upper boundary of the AR5 "likely" range should not be misconstrued as a worst-case upper limit, as was done in Kerr's story as well as elsewhere in the media and blogosphere. For policy and planning purposes, it may be necessary to adopt particular numbers as an upper

limit, but according to our assessment, the current state of scientific knowledge cannot give a precise guide.

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Credit for Impact Theory

IN THE NEWS FOCUS STORY "IMPACT THEORY gets whacked" (11 October, p. 183), D. Clery summarizes the present conundrum we face in understanding the Moon's eerie isotopic similarity to the Earth. However, it contains one oversight in ascribing the proposal of the giant impact hypothesis to William Hartmann and Donald Davis (*I*) in 1975. In actuality, two groups developed this idea contemporaneously and both discussed essentially the same idea at a Cornell conference in 1974, at which all four researchers were present. Hartmann and Davis directly acknowledged this in a footnote in their paper.



CREDIT: ANDREW MANDAMAKER/WIKIMEDIA COMMONS

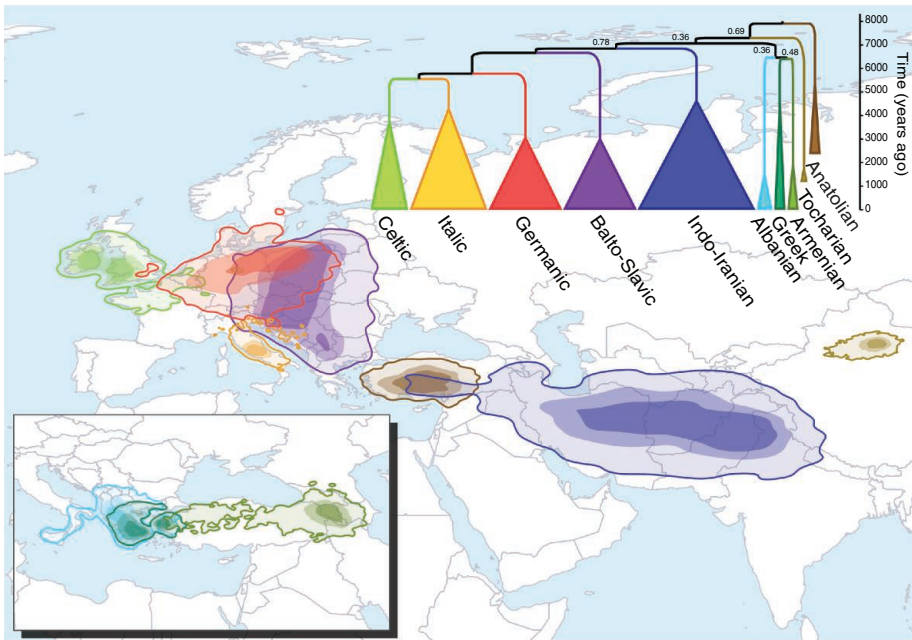
CORRECTIONS AND CLARIFICATIONS

Reports: “Anthropogenic seismicity rates and operational parameters at the Salton Sea geothermal field” by E. E. Brodsky and L. J. Lajoie (2 August, p. 543, published online 11 July 2013). There are two typographical errors in Table 1: The reported phase lag in the time interval of 1982–1991 associated with injection should be 0 instead of 6. Also, the correlation between injection and seismicity in the 1991–2006 time window should be 0.25 instead of 0.26. The HTML and PDF versions online have been corrected.

Reports: “Mapping the origins and expansion of the Indo-European language family” by R. Bouckaert *et al.* (24 August 2012, p. 957). The authors are grateful to William Chang and Andrew Garrett for informing them that there was a problem with the data matrix they used. The error occurred when 13 languages were removed from the original 116-language data matrix (<http://ielex.mpi.nl>) because they were colonial varieties or doculects, for which the authors had a better source. Removing these languages produced 283 “empty” columns of zeros (out of 6279), which the authors neglected to omit. Columns full of zero entries can potentially bias rate estimates from model-based phylogenetic inference. In addition, this revealed an error in the ascertainment bias correction for all-zero columns in the BEAST code [A. J. Drummond, A. Rambaut, *BMC Evol. Biol.* **7**, 214 (2007)]. The authors have therefore rerun the analyses with corrected data and BEAST code. The covarian model is now the best-fitting model of cognate evolution [C. Tuffley, M. A. Steel, *Math. Biosci.* **147**, 63 (1998); D. Penny *et al.*, *J. Mol. Evol.* **53**, 711 (2001)]. Under this model, the basic inference about the geographic origins of Indo-European remains unchanged (revised Table 1 shown below); however, the tree topology differs slightly (revised Fig. 2 shown below) and date estimates are younger, although still showing a better fit with the Anatolian hypothesis than with the Pontic steppe hypothesis (median = 7579 years BP; 95% HPD interval = 5972 to 9351 years BP). The date ranges under the different models of cognate evolution, including the previously best-fitting model (the stochastic-Dollo), are shown in a newly added fig. S13. Revised supplementary material with revised versions of all affected tables and figures, as well as updated xml code, has been posted online. Two points in the main text also need correcting. First, the analysis, in which the authors constrain the tree topology to fit with an alternative pattern of diversification, still shows strong support for an Anatolian origin, but the Bayes factors are slightly different ($BF_{\text{Steppe I}} = 174.02$, $BF_{\text{Steppe II}} = 145.35$). Second, in the revised analysis, the five major Indo-European subfamilies—Celtic, Germanic, Italic, Balto-Slavic, and Indo-Iranian—all emerged as distinct lineages between 4000 and 7000 years ago, not between 4000 and 6000 years ago as previously stated.

Phylogeographic analysis	Bayes factor	
	Anatolian vs. steppe I	Anatolian vs. steppe II
RRW: All languages	380.4	625.2
RRW: Constrained	174.0	145.4
RRW: Ancient only	828	+ ∞
RRW: Contemporary only*	73	+ ∞
Landscape aware: Diffusion	161.10	79.14
Landscape aware: Migration from land into water less likely than from land to land by a factor of 10	63.0	31.2
Landscape aware: Migration from land into water less likely than from land to land by a factor of 100	120.3	59.0
Landscape aware: Sailor	119.4	59.6

*We note that although this analysis appears to show strong support for the Anatolian theory, this is because the Kurgan homeland was never sampled, whereas a small number of samples fell within the Anatolian range. This is perhaps not surprising given the absence of Anatolian and Tocharian languages from this analysis.



Hartmann and Davis were first to publish. They advocated that Earth collided with a sublunar mass object near the end of its formation, based on models of the Earth’s assembly. If the Earth’s core had formed at the time of the collision, they argued, ejected material would be depleted in iron, thus offering a natural explanation for the Moon’s low density. Cameron and Ward’s work appeared in early 1976 (2). They recognized two additional (and critical) aspects of the problem: First, a mechanism to alter ballistic trajectories (such as vaporization and resulting pressure gradients) would be required to allow ejected material to go into orbit around the Earth. Second, the impact scenario implies a Mars-sized impactor, based on matching the anomalously large angular momentum of the Earth-Moon system.

Given this, most of the Moon origin community attributes the impact theory jointly to both Hartmann and Davis (1975) and Cameron and Ward (1976).

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Social Security and Medicare in the Black

IN HIS NEWS & ANALYSIS STORY, “U.S. SHUT-down ends, but not budget anxiety” (25 October, p. 410), J. Mervis writes, “Hunter Rawlings, president of the... Association of American Universities,...says one major impediment to increased science spending is the continued growth of so-called entitlement programs, such as Social Security and Medicare.” Social Security is paid for by a tax dedicated to Social Security. Medicare Parts A and B are also paid for by a tax dedi-

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

cated to Medicare. Although future problems loom, to date both programs have run in the black through the course of their history (1). In other words, neither Social Security nor Parts A and B of Medicare have contributed a penny toward the current U. S. deficit. On the contrary, the discretionary side of the budget has borrowed heavily from them.

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Mercury Toxicity in Children

AS SCIENTISTS AND PEDIATRICIANS WHO study the impacts of toxic chemicals on children's health and deal with the consequences of exposure, we are concerned that in her Editorial "Mercury and health" (27 September, p. 1430), M. McNutt suggests



that more research is needed to determine the effects of sublethal doses of mercury on the development of young children.

The developmental toxicity of mercury has been studied extensively for more than two decades (1–10). A major review by the National Academy of Sciences (1) concluded that evidence for the developmental neurotoxicity of methylmercury is strong and highly credible, even at low levels of exposure. These findings provide critical support for the Minamata Convention. The Editorial's call for additional research, at a time when abundant scientific evidence already exists, may delay full ratification of the Minamata Convention. Unnecessary calls for additional research have been a

major factor in long delays in recognition, remediation, and reparations to the victims at Minamata.

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HISTORY OF MEDICINE

Epistemology in the Time of Cholera

John Harley Warner

For half a century, Charles Rosenberg's *The Cholera Years (1)* has stood as a foundational model for the history of medicine. Exploring medical, religious, and civic responses to successive epidemics, Rosenberg traced the transformation of American medicine and society alike. In the ensuing decades, the theoretical tool kit that scholars use in analyzing the history of disease and medicine has been remade. It is therefore with considerable anticipation that one opens *Knowledge in the Time of Cholera*, a book that revisits pivotal moments in the history of cholera deploying the newer historiography of the professions and analytical methods of the sociology of knowledge.

But historical sociologist Owen Whooley (University of New Mexico) has still larger ambitions, and he retells the familiar story of the professionalization of American medicine by placing epistemological struggle front and center. He takes to task Paul Starr's *The Social Transformation of American Medicine (2)*, still the premier account of professionalization, charging that it perpetuates a "truth-wins-out" narrative in which scientific progress drives changes in cultural authority. Instead, drawing insight from the sociology of knowledge into how actors struggle to win recognition for their ideas—here, competing claims about what counts as legitimate medical knowledge—Whooley examines debates over professional authority through the lens of competing epistemological allegiances. Cholera epidemics provide a sampling device but are also scripted as a leading cause of change.

Cholera first struck the United States in 1832, killing swiftly and rendered more terrifying by the medical disagreements about how to prevent, treat, or explain the new disease. Alternative healers such as Thomsonians and homeopaths seized upon this confusion to mount an attack on regular physicians' ideas, practices, and epistemological presuppositions. Glossing over earlier epistemological battles, Whooley

locates in the 1832 epidemic the origins of an epistemic contest that would persist across the century. He recounts the familiar story of how alternative healers—proselytizing for

a democratic vision of medicine—lobbied successfully for the repeal of all licensing laws, making the American medical marketplace the most open in the Western world. Whooley relies too heavily on a caricature of elitism drawn from the polemics of alternative critics, even adopting the label "allopath"—an epithet of derision coined by homeopaths—to describe mainstream physicians who called themselves "regular." But he is right in underscoring the cardinal place of epistemological controversy in battles over professional authority and bids for public trust.

Cholera struck again in 1849, and uncertainty about how to explain or stem the epidemic persisted. Homeopathy had grown powerfully competitive, intensifying the epistemic crisis. Regular physicians, Whooley suggests, responded to this assault in two ways. First, after 1849, they turned away from rationalism to embrace the radical empiricism of the Paris School, seeking a more resilient epistemological foundation for their

knowledge claims. Second, they founded the American Medical Association (AMA) as "an organizational response to the epistemological problem of adjudication," barring homeopaths from membership and creating the appearance of cohesion. Timing and causality are a bit confused here: The turn to Parisian empiricism occurred by the early 1830s, not the late 1840s, and the AMA was founded in 1847, before the 1849 epidemic. But the larger point that these maneuvers should be understood partly as responses to an ongoing epistemological crisis remains cogent.

The most intriguing chapter is anchored in German bacteriologist Robert Koch's 1884 announcement that he had discovered the microbial cause of cholera. Whooley shows how both homeopaths and allopaths staked a claim to Koch's work. Their competing discovery narratives had the dual aims of converting colleagues and claiming Koch as their own. The allopathic narrative proved more "effective," giving regular physicians possession of not only Koch but also the laboratory and, hence, a new and ultimately compelling epistemological foundation for regrounding medicine. To consolidate ownership, Whooley argues, allopaths began to build networks with German laboratory science. For Americans going abroad, "it was not until the emergence of bacteriological findings that Germany came to be seen as an alternative to Paris"—an engaging suggestion were it not that the shift from France to Germany by Americans studying experimental physiology and new clinical specialties began not in the mid-1880s but in the 1860s and 1870s.

Bacteriological analysis played little role in managing cholera when it last struck in 1892, but the laboratory nonetheless gave regular physicians a platform for epistemological closure. Whooley recounts how reformers went on to remake American medicine—conceptually and institutionally—with the laboratory as its core, scotching the power of alternative criticism and consolidating professional authority. "By the end of World War I, the reforms of allopathic medicine were complete, and the organizational infrastructure and the modern medical epistemology we know today were more or less in place."

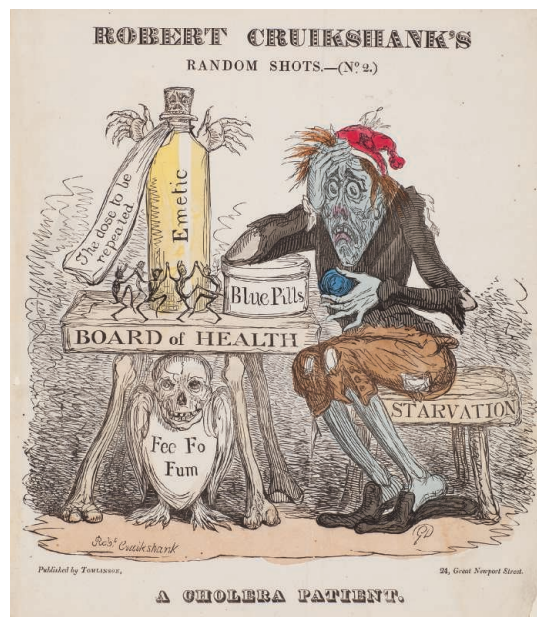
Knowledge in the Time of Cholera is a provocative book, sweeping in scope and valuable for bringing the interpretive insights of the sociology of knowledge to bear on 19th-century medicine. Too often

Knowledge in the Time of Cholera

The Struggle over American Medicine in the Nineteenth Century

by Owen Whooley

University of Chicago Press, Chicago, 2013. 321 pp. \$90. ISBN 9780226017464. Paper, \$30, £21. ISBN 9780226017631.



A British perspective.

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the author fences with straw figures: to exemplify “conventional histories” of Koch’s discoveries, for example, he skips over three-quarters of a century of scholarship to narratives written in the 1920s and 1930s. And one would never suspect from this book that other historical accounts of 19th-century American medicine have placed epistemology center stage. Nevertheless, Whooley does important work by drawing attention to epistemic contest as key to understanding the emergence of modern medicine. If the book succeeds in infusing a sensibility to epistemology into sociological analysis of medical professionalization, it will make a welcome contribution indeed.

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NATURAL HISTORY

Our Many Ties to Feathered Fauna

Henry T. Armistead

A weighty 2.5-kg tome, *Birds and People* provides an engaging overview of the relationships between humans and birds, a broad subject encompassing topics from advertising, agriculture, and aircraft collisions, through hunting and literature, to veterinary science. The work falls short of exhaustive, but “to do full justice to the relationships between birds and people worldwide one would easily fill 20 volumes of this size.” That said, the text and nearly 400 splendid color photographs do justice enough to a vast subject. Mark Cocker, an accomplished ornithologist and writer, spent 17 years researching it. He tapped 650 consultants across 81 countries, and photographer David Tipling traveled to 39. Their efforts are thoroughly referenced by circa 2575 source notes.

Relationships between people and birds have been explored in many previous books. These often concentrate on a discrete aspect, such as birds of the Bible, Shakespeare’s birds, birds versus agriculture, falconry, and parrots as pets. Others are organized around a series of such themes. *Birds and People* is arranged phylogenetically, with sections on



Hunting partners. Kazakh nomad Dal Han and his golden eagle, which “caught all the foxes in his fur coat.”

144 extant families (out of just over 200) and two extinct ones. (Families that have “limited cultural profiles” or whose cultural links overlap with several other families are simply listed.) Within each family account, particular themes and individual species names are flagged in arresting red print. Comments on individual species range from a single sentence to the nine-plus pages devoted to the obvious, ubiquitous, ordinary chicken, originally (and still) the red junglefowl (*Gallus gallus*) in the wild, and its overriding importance. (Cocker notes that with a global total of at least 12 billion chickens, the species is now “the most numerous bird on the planet.”)

One of the book’s strengths is its documentation (through text and photography) of the importance of birds to tribal people—for example, the ornamental headdresses of New Guinea tribesmen composed of feathers from birds-of-paradise. Another recurrent theme is conservation, which reflects the considerable input from members of the BirdLife International network. Surveying literature, music, and film, Cocker mentions Keats’s nightingale, Shelley’s skylark, Hitchcock’s birds, Coleridge’s albatross, and some avian songsters of Mozart and Tchaikovsky. He did not find room to include Hardy’s “darkling thrush,” Yeats’s swans and falcon, Poe’s raven, Brăncuși’s “bird in space,” or various popular songs such as “Rockin’ Robin.”

Despite a “Select Bibliography” running to nearly 750 entries, some important titles on the book’s theme go unmentioned. These include Waldo McAttee’s writings on the economic impact of birds as well as works by R. K. Murton and Robert Welker (1, 2). And although the esteemed English nature writer William H. Hudson gets big play with five of his books listed, his classic *Birds and Man* (3) is not among those.

Impressive as most of the book is, one wonders about the value of the skimpy glossary (14 terms) and set of biographical sketches (covering only a dozen individu-

als). And I would have preferred a single index over the separate species and general versions. In some species accounts, Cocker strays from discussing relationships with people, concentrating instead on interesting aspects of the bird’s biology. But considering that sooty terns (*Onychoprion fuscatus*) spend years

aloft without alighting, that some bar-tailed godwits (*Limosa lapponica*) fly nonstop for seven days to cover the 11,000 km from Alaska to New Zealand, or that blackpoll warblers (*Setophaga striata*), weighing about what a first-class letter does, fly nonstop from New England or the maritime provinces to South America, who will quibble over occasional, brief departures from the book’s main theme?

Perhaps emblematic—and certainly more charismatic than most—of birds’ relationships with humans is falconry. In this spirit, the spectacular cover shows four mounted nomad Kazakhs, each bearing a golden eagle (*Aquila chrysaetos*) on his right arm. The Holy

Roman Emperor Frederick II’s *De arte venandi cum avibus* (*The Art of Hunting with Birds*), which appeared in the 13th century, is still revered by falconers. Hundreds of books on falconry have followed. Roger Tory Peterson, whose field guides kindled so many

people’s interest in birds, once noted: “Man has emerged from the shadows of antiquity with a Peregrine on his wrist. Its dispassionate brown eyes, more than those of any other bird, have been witness to the struggle for civilization, from the squalid tents on the steppes of Asia thousands of years ago to the marble halls of European kings in the seventeenth century” (4).

The Kazakhs and Frederick receive their due in *Birds and People*. Alas, Peterson does not. But this is a small matter in light of the book’s myriad other virtues. Well laid out and designed and fluidly written, the book seems the best yet on its complex subject.

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Birds and People

by Mark Cocker;
photographs by David Tipling
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ISBN 9780224081740.

Open Learning at a Distance: Lessons for Struggling MOOCs

Patrick McAndrew* and Eileen Scanlon

Support for nontraditional students, team-based quality control, and assessment design are critical.

Free education is changing how people think about learning online. The rise of Massive Open Online Courses (MOOCs) (1) shows that large numbers of learners can be reached. It also raises questions as to how effectively they support learning (2). There is a time-liness in the introduction of MOOCs, reflecting the right combination of online systems, interest from good teachers in reaching more learners, and banks of digital resources, predicted as a “perfect storm of innovation” (3). However, learning at scale, at a distance, is not a new phenomenon. Seeing MOOCs narrowly as a technology that expands access to in-classroom teaching can miss opportunities. Drawing on decades of lessons learned, we set out aims to help spur innovation in science education.

Education based on gathering people together into a physical location is limited to those who can afford it and who make it past the filters that attenuate participation in higher levels of education. Those filters are inevitable on cost grounds; to meet global needs “would require four major campus universities ... to open every week” (4). The arrival of MOOCs highlights that there are alternatives. With courses enrolling over 100,000 students, MOOCs can reach students who have breaks in study, change where they study, mix study with work, and take at least part of their study online. Such students are now the majority, forming more than 70% of those in U.S. post-secondary education (5).

Recommendations for Open Learning

We ought not behave as if learning at scale is unexplored territory and that there is no previous experience in being massive, open, or even online, upon which to build. Distance universities, such as The Open Univer-



sity (OU) established in Britain more than 40 years ago, from their inception, ran courses for thousands of learners, accepted open entry, and led the move into online methods of teaching and learning. In each case, they provide lessons likely to apply in the new context of MOOCs.

Build on distance-learning pedagogy. Some of the steps taken toward “massive” classes simply follow the observation that a lecture presented to a few hundred students can be viewed by many more once put on the Web. But numbers of views and downloads of PowerPoint do not mean learners have engaged. Effective distance-learning pedagogies that lead the learner through tasks have been applied across all subject domains at scales that cannot be achieved in face-to-face classes. A classic challenge for distance learning is “could you teach surgery?” The University of Edinburgh now does just that (6). Support built into OU materials, together with support from tutors and assessment, has enabled 1.6 million people (7) to complete university level courses without the need to meet initial entry requirements. Teaching at a distance combines media to motivate and enthuse, including television programs broadcast through the BBC, experiment kits both physical and virtual, and online simulations and case studies. “Exploring Science” introduces science to 4000 students each year with virtual field trips, and the Open Science

Laboratory builds a collection of tools to combine remote access, virtual experiments, and citizen science (8) into the curriculum.

Advice: Interactions between student-teacher, student-student, and student-materials all can act to support learners (9). Paying attention to the content, and building materials that do the teaching (10), allows direct contact between teacher and learner to be reduced. Structured tasks guide the learner. Working online offers the chance to build in interactivity. Pre-

sentation using video or broadcast is adjunct and motivates; it is not the core. On the other hand, carefully constructed text-based material can feel to the student as if it is speaking to them. Then, using multimedia can build further ways to engage learners in science.

Plan to help learners who need support. “Open” is not the same as “free.” Openness means accepting those who want to learn as well as those ready to learn. Learning is challenging, so helping students is essential. Some people will manage on their own, but that is not enough for genuinely inclusive education. The self-paced, location-independent properties of online learning make it attractive to the marginalized and those with disabilities (11). Rapid fall-off identified in many MOOCs (12), where only 10% of those who register may complete the course, reflects retention challenges. How we approach support for learners influences retention. Early contact with a tutor prevents drop out, and student attitudes toward the tutor matter (13). For large-scale operation, tutors focus on effective and timely feedback to learners. Support is particularly important as activities start; submission of the first assignment predicts eventual success with a course.

Advice: A vital step in coping with accessibility is to recognize the importance of support and the feeling of being supported. The human touch can operate at a distance. For example, the Mechanical MOOC allows

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(semi-)automated contact. Synchronous events and analytics can let learners know how they are performing. Assisting those who have difficulty learning requires skills and is hard to achieve at no cost; focus attention on initial support and feedback for great-est benefits.

The power of well-designed assessment. Once online, assessment often becomes the main driver and route for feedback to students, offering pacing and control. With the appropriate approach to assessment, online education need not be a lonely activity.

Distance universities, from their inception, ran courses for thousands of learners, accepted open entry, and led the move into online methods of teaching and learning.

Rather, it offers ways to work collaboratively and benefit from peer interaction (14) and offers scope to take part in real-world activities. Such authentic assessments (15) can also provide evidence that can be shared. For example, the Evolution MegaLab and Cosmic Genome Project provide both learning opportunities and new sources of data across a large, global scale.

Advice: Place as much importance on designing the assessment as developing instructional content. Leading with questions and helping learners understand what makes a good answer will shape their approach to learning and path through content. Build in responses that give feedback designed to ensure understanding, and feed-forward that helps the learner face the next challenge. Marking achievements—for example, with badges (16)—gives ways for learners to value the experience; even if they cannot continue, there is no need for them to feel that they have failed. Learners can be encouraged to act as real scientists. Recognizing and supporting the increases in skills and gains in reputation that they make in the science community will then help them engage and sustain interest.

Ensure quality by working together. Building courses in the open means that mistakes happen in public. Quality assurance is essential. One technique for quality, imported by the OU from a long-term relationship with the BBC, is that material is “fit for broadcast,” applying editorial consistency measures and validation of content. Another technique, a multidisciplinary team approach, ensures scrutiny from a range of views, with media and educational technology specialists who design the learning experiences working alongside academic specialists who

bring the knowledge of science teaching. As technology or pedagogy is added, it is tested and checked against usability and accessibility requirements. These aspects are incorporated into the design and approval process (17). The team-based approach is being recognized by those from campus-based roots, including innovative approaches to include graduate students in development (18).

Advice: Quality measures are expensive and under pressure to change as courses become shorter and the half-life of latest information and tools is reduced. The overall

model, though, is robust: Set quality levels, work in teams, and test before your learners do. These can be adjusted, with increased speed leading to increased risk. Steps to simplify content, minimizing rather than removing quality checks, and allowing feedback after release, help speed the process (19).

The Future for Open Education

Classic models of education cannot meet all our needs. Simply transferring those models online is not the most effective approach to open education. We need new approaches that can operate at low cost in the open. The current generation of MOOCs are already providing some benefits, their global reach finding enthusiastic learners. But MOOC providers have been criticized for their elite model, lack of reliability, low proportions of learners completing courses, and overall pedagogy (20).

Distance education has tackled the challenges of learning science at scale through techniques such as simulated practical work and access to remote laboratory facilities. The emergence of “citizen inquiry” activities is promising, leading to ideas of crowd learning (21), combining elements of inquiry learning and cyberscience (22). The challenges are to make such opportunities for informal learning bring lasting benefits.

Our advice on implementation of open online courses should help build large-scale open learning. Completely open operation online also brings new aspects. For example, using effective open licenses, such as Creative Commons, allows us to share the ways we develop teaching, as well as giving clear permissions to learners. We need to study these new contexts to find out more about

motivations for participants, how to scale up to genuinely massive access to learning, and how best to assess learning. The opportunity for experimentation gives us the chance to learn more ourselves, as well as to educate others.

Distance universities, from their inception, ran courses for thousands of learners, accepted open entry, and led the move into online methods of teaching and learning.

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EVOLUTION

How Cooperation Defeats Cheats

Claire N. Spottiswoode^{1,2}

In the spring of 1879, Hermann Lau shot two white-winged choughs, *Corcorax melanorhamphos*, off their nest in Queensland, Australia. He watched as additional choughs continued to attend the nest, proving that a cooperative group shared parental care (1). Since then, cooperatively breeding birds have had a starring role in efforts to explain the evolution of complex animal societies. We now know that “helpers-at-the-nest” who forgo reproduction are often relatives of the breeding pair. Genetic payoff is, thus, one of several advantages that helpers can gain from their superficially altruistic behavior (2). On page 1506 of this issue, Feeney *et al.* (3) show that collective defense against brood parasites (see the figure) can enhance such benefits of cooperation.

Why do some bird species cooperate and others do not? Global analyses have shown that cooperative breeding (now known from 9% of species) is associated with a slow pace of life (characterized by high survival rates and low turnover of breeding territories) (4), monogamy (which facilitates kin selection within families) (5), and unpredictable environments (such as arid zones) that might favor cooperation as a bet-hedging strategy (6). But these factors often fail to predict the incidence of cooperation among related species or within geographical regions (7).

Feeney *et al.*'s study is built on the premise that brood parasitism—reproductive cheating by species such as cuckoos and cowbirds, which exploit other birds to raise their young—is a severe selection pressure on their hosts' breeding strategies. Parasitized parents typically not only lose their current offspring but also waste a whole breeding season raising a demanding impostor. The best way to avoid parasitism is to repel adult parasites from the nest. Feeney *et al.* show that sociality can be pivotal to this process.

The authors begin by unfolding a new map. Using data compiled by BirdLife International, they show that the global distribution of cooperatively breeding birds overlaps strikingly with that of brood parasites. This overlap need not reflect a causal relationship:



Cooperation in action. As a female, parasitic lesser honeyguide (*Indicator minor*) attempts to lay her egg in the nest-hole of black-collared barbets (*Lybius torquatus*), she is violently repelled by the host birds (11). Photos taken in Grahamstown, Eastern Cape Province, South Africa.

The same unpredictable environments that favor cooperation could also favor alternative breeding strategies such as parasitism. However, the authors go on to show that even within geographical regions rich in both parasites and cooperators—Australia and southern Africa—cooperative breeders are much more likely than noncooperative species to be targeted by brood parasites.

To determine the reasons for this correlation, Feeney *et al.* studied cooperative breeding in superb fairy-wrens (*Malurus cyaneus*) in Australia. Horsfield's bronze-cuckoos (*Chalcites basalus*) should benefit from targeting larger groups of fairy-wrens because more helpers mean faster chick growth. Yet, data from a 6-year field study show that in practice, cuckoos rarely experience this advantage, because larger groups of fairy-wrens much more effectively detect and repel egg-laying intrusions by cuckoo females, mobilizing group defenses with a cuckoo-specific alarm call.

Thus, cooperation and parasitism could reciprocally influence one another: Cooperators might be more attractive targets because they make better foster parents, but once

Brood parasites may play a key role in the evolution and maintenance of cooperative breeding in birds.



exploited by parasites, they are also better able to fight back, helping cooperation to persist (8). Feeney *et al.* find that superior anti-cuckoo defenses in larger groups account for 0.2 more young fledged per season on average than smaller groups—a substantial boost given the fairy-wrens' low annual fecundity.

These results show convincingly that defense against brood parasites augments the benefits of helping, promoting the persistence of cooperation. But as the authors note, they cannot reveal what caused cooperation to evolve initially. Brood parasitism alone cannot resolve the question of why some birds breed cooperatively. For example, cooperative kingfishers and bee-eaters are heavily parasitized in Africa but not in Australasia, showing that other advantages of helping behavior are sufficient for cooperation to persist. But we should take parasitism seriously as an important force in a cooperative life. Indeed, it may provide a mechanism contributing to the previously discovered global correlates of cooperation (4–6).

Some insight into the likely order of evolution might come from further comparative predictions. For instance, if cooperation arose

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first as a defense against parasitism, cooperators may be most prevalent among hosts that rely heavily on repelling adult parasites, rather than on antiparasite strategies at later reproductive stages, such as egg or chick discrimination (9). In contrast, if parasites target existing cooperators because they provide superior care, this should be especially true of parasites whose chicks have the most pressing needs—for instance, those in parasitic families with large body size relative to their hosts or those whose chicks do not kill host young and therefore must share their foster parents' care.

Could there be a similar association between cooperation and parasitism among other highly social animals? Cooperation in mammals clearly persists irrespective of parasitism, given that there are no known

brood-parasitic mammals (perhaps because it would be difficult for a mammal to insert live young into another's care). But repelling parasitic egg-laying intrusions is crucial to many hosts of socially parasitic insects and has shaped sophisticated adaptations and counterdefenses for and against brute force and secrecy (10). It will be fascinating to explore how selection for antiparasitic defense has interacted with monogamy and defensible resources as forces favoring kin-selected cooperation in invertebrates, touching on an active debate in evolutionary biology.

Answers to such comparative questions will ultimately be limited by our knowledge of natural history. The work by Feeney *et al.* is testament to the evolutionary insights enabled by careful long-term field studies, together with the cumulative legacy of those

naturalists who made the unglamorous effort to record and publish observations of real animals in real places.

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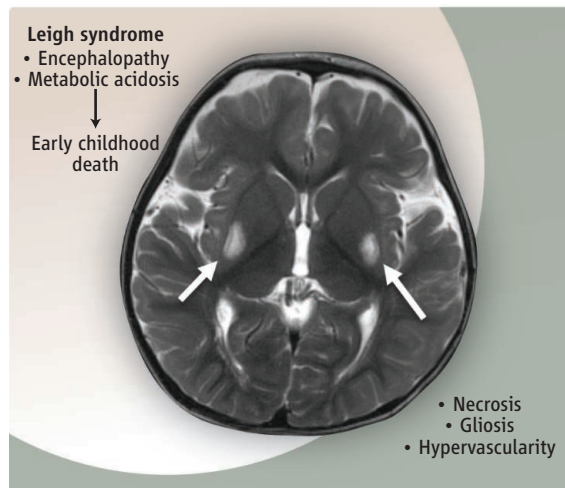
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MEDICINE

A Common Pathway for a Rare Disease?

Scott B. Vafai^{1,2,3} and Vamsi K. Mootha^{1,2,3}

Leigh syndrome is a fatal, infantile neurodegenerative disease first described more than 60 years ago (1). Children with Leigh syndrome typically are born with normal prenatal development, but decline after intermittent episodes of encephalopathy and metabolic acidosis, leading to death within the first few years of life. The diagnosis is based on magnetic resonance imaging of the brain, which reveals bilaterally symmetric lesions in the brainstem and basal ganglia (see the figure) that correspond to regions of necrosis, gliosis, and hypervascularity, with relative sparing of neurons in the early stages of the disease. At present, no effective therapies are available for Leigh syndrome, and the mainstay of management is supportive care. On page 1524 of this issue, Johnson *et al.* (2) demonstrate that rapamycin, a compound that inhibits a protein kinase called mechanistic target of rapamycin (mTOR), delays the onset and progression of neurological symptoms in a mouse model of Leigh syndrome. mTOR lies at the hub of cellular signaling,



Magnetic resonance imaging of Leigh syndrome. A characteristic of Leigh syndrome, an early childhood neurological disorder, is bilateral lesions in the basal ganglia (arrows showing the putamen region of the brain).

sensing nutrient availability to regulate protein translation, autophagy, and metabolism. The new connection to mitochondrial disease widens our view of the signaling pathway, with potential therapeutic implications.

Leigh syndrome is a prototypical mitochondrial disorder (3), and causal mutations have been described in more than 40 genes

A rare childhood disorder caused by mitochondrial dysfunction is treated by the drug rapamycin in a mouse model of the disease.

required for mitochondrial production of adenosine triphosphate (ATP), notably oxidative phosphorylation. Johnson *et al.* noted that glucose restriction, an intervention that works in part through the TOR pathway (4), extends the life span of yeast lacking homologs of genes implicated in Leigh syndrome. Motivated by this observation, the authors administered daily intraperitoneal injections of rapamycin to a mouse model of Leigh syndrome. These animals recapitulate many of the features of the human condition, including bilateral brainstem lesions, breathing abnormalities, and premature death (5). Specifically, they lack the NADH dehydrogenase (ubiquinone)

Fe-S protein 4 (NDUFS4), a component of complex I of the mitochondrial oxidative phosphorylation system. Rapamycin treatment partially rescued the mortality phenotype in these mice, more than doubling their median life span. Equally striking, though, is that rapamycin alleviated the development of brain lesions and attenuated the increase in

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the concentration of lactate (a by-product of pyruvate metabolism) in the brain.

The mechanism by which rapamycin delays progression of the disease in the mouse model is not clear, but Johnson *et al.* exclude key possibilities and provide several intriguing clues. Treatment with the immunosuppressive drug tacrolimus showed no protective effect, arguing against the known immunosuppressive action of rapamycin as the sole basis. Rapamycin treatment has been shown to benefit cellular models of disease due to mitochondrial DNA mutations through autophagic clearance of defective mitochondria (6, 7). However, the authors observed no improvement in the function of the oxidative phosphorylation system, indicating that the underlying biochemical lesion had not been improved through increased organelle turnover or other mechanisms. Rapamycin treatment can trigger the mitochondrial unfolded protein response (8), an adaptive retrograde response through which mitochondrial stress signals to the nucleus to increase expression of genes that improve mitochondrial protein homeostasis. However, Johnson *et al.* did not see induction of this response in the brain. The authors found that mTOR complex 1 (mTORC1), one of the two multisubunit complexes that contain mTOR, is activated in the brains of *Ndufs4*-deficient mice, and its inhibition with rapamycin conferred neuroprotection. This suggests that mTORC1 activation may contribute to disease pathogenesis in Leigh syndrome. Why mTORC1 is activated, though, is not clear, and determining this should provide valuable insights and additional therapeutic strategies.

Regardless of the mechanism, rapamycin is already approved by the U.S. Food and Drug Administration, which makes the current study quite exciting. However, additional preclinical studies are required given that the drug is not without its risks, and numerous practical questions remain. Rapamycin is used in the pediatric population for posttransplant immunosuppression and treatment of tuberous sclerosis (a tumor syndrome due to loss-of-function of negative regulators of mTOR). The immunosuppressive effects of rapamycin must be kept in mind, because infections can trigger clinical deterioration in Leigh syndrome and other mitochondrial encephalopathies. There are more than 40 genetic causes of Leigh syndrome, with an estimated incidence of 1:40,000 children (9), and at present, it is not known whether mTOR inhibition would be useful in all cases of Leigh syndrome, in cases of complex I deficiency, or only in

cases of *NDUFS4* deficiency. In addition, Johnson *et al.* treated the *Ndufs4*-deficient mice with rapamycin regularly from the time of weaning and clearly demonstrate efficacy. In practice, though, most cases of Leigh syndrome present with acute crises, and it is unclear whether rapamycin can reverse existing neuropathologic lesions, or slow their progression once begun.

Although it is premature to initiate human clinical trials on rapamycin treatment for mitochondrial disorders, a rapid and constructive path forward can be defined. The most important next step is to replicate the findings of Johnson *et al.* in independent laboratories. It will be crucial to evaluate the utility of rapamycin in mouse models after the onset of neurological symptoms, to more realistically mimic the human scenario. The dose of rapamycin used by Johnson *et al.* achieves concentrations in the blood that are above the target range in patients, so the drug's efficacy at a clinically relevant dose should be tested in future studies. Rapamycin also should be evaluated across animal models of other mitochondrial disorders (10) to more accurately define the patient population that may benefit. Moreover, rapamycin is an allosteric inhibitor of mTORC1 that does not completely inhibit its catalytic activity, so treatment with newer mTOR inhibitors should be attempted, as they might prove more effective. These studies, which could be completed quickly, will guide the

design of rigorous clinical trials to evaluate efficacy and safety (11).

The study by Johnson *et al.* is particularly important because it points to a link between a well-studied cell signaling pathway, for which existing drugs are available, and the pathogenesis of a devastating but prototypical mitochondrial disorder. Rapamycin has proven effective in mouse models of Parkinson's disease and Alzheimer's disease, and extends life span in a number of species (4). The precise basis of these broad protective effects remains unclear, though they may provide additional evidence for a mitochondrial contribution to age-related disorders.

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MICROBIOLOGY

No Barriers to Cellulose Breakdown

Alex Berlin

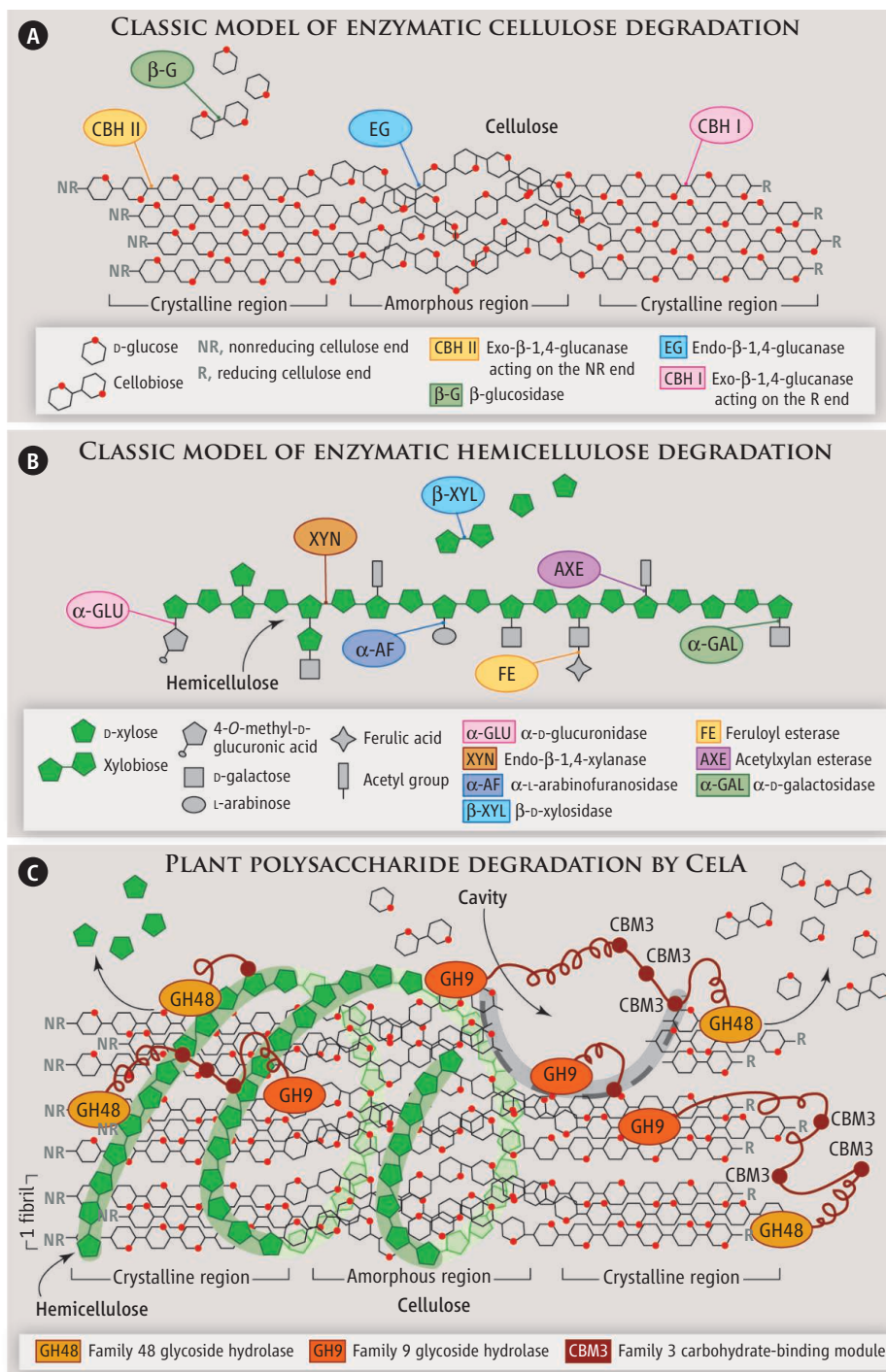
A bacterial enzyme efficiently breaks down cellulose and hemicellulose without the help of other enzymes.

One of the most promising routes to sustainably produced liquid fuels is the enzymatic conversion of biomass. Efficient enzymatic conversion of biomass into fermentable sugars requires the concerted action of optimally balanced mixes of glycoside hydrolases and accessory proteins (1, 2). On page 1513 of this issue, Brunecky *et al.* (3) show that CelA, an unusually large secreted multidomain cellulase from the thermophilic bacterium *Caldicellulosiruptor bescii*, efficiently breaks

down microcrystalline cellulose, outperforming enzymes typically used commercially to break down biomass.

Acting alone or in combination with a β -glucosidase, CelA breaks down highly recalcitrant cellulose faster than does an enzyme mixture commonly used in commercial biomass enzyme products. Furthermore, CelA shows higher specific activity on raw biomass than on physicochemically pretreated biomass. These results may be a turning point in the development of future bioconversion technologies that do not require biomass pretreatment or the participation of a large number of enzyme components.

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How plant polysaccharides are broken down. (A)

The enzymatic degradation of cellulose includes the joint action of exoglucanases or cellobiohydrolases (CBHs), endoglucanases (EGs), and β -glucosidases. CBHs cleave cellulose in a processive manner, releasing primarily cellobiose from the ends of the cellulose chain. Unlike CBHs, EGs can act on both cellulose and hemicelluloses (12). The degradation of cellulose by the joint action of CBHs and EGs is the backbone of today's commercial cellulase products. (B) Hemicellulose degradation also involves the synergistic action of diverse enzyme activities (4). The concerted action of a myriad of hemicellulases efficiently hydrolyzes hemicellulose. The removal of the hemicellulose barrier enhances cellulase activity on biomass cellulose by increasing the accessible cellulose surface area. (C) As Brunecky *et al.* report, *C. bescii* CelA and its fragments can depolymerize biomass polysaccharides to glucose, cellobiose, and xylose via a combined surface ablation and cavity-forming mechanism without the help of accessory proteins. CelA comprises a glycoside hydrolase family 9 and a family 48 catalytic domain, as well as three type III cellulose-binding modules.

biomass enzymes. These novel catalytic enhancers differ dramatically in mode of action from classic cellulases and hemicellulases. For instance, certain lignin oxidases, such as laccases, can boost cellulase activity on biomass cellulose, possibly by releasing cellulases from their nonproductive binding sites on biomass lignin (5, 6) and thereby increasing the effective concentration of free cellulases in solution.

Copper-dependent lytic polysaccharide monooxygenases (LPMOs) also boost cellulase activity (7). Unlike cellulases and hemicellulases, LPMOs require the presence of electron donors, water, and oxygen to degrade cellulose (8). The unusual oxidative mechanism of action of LPMOs on cellulose helps to accelerate the reduction of biomass recalcitrance and to increase the accessibility of biomass polysaccharides to exo- and endoglucanases.

Cellulase activity can also be boosted by the addition of noncatalytic proteins. Such proteins promote cellulase activity presumably by either reducing the nonproductive binding of cellulases (9) or by increasing their accessibility to cellulose via reduction of cellulose crystallinity (swollenins) (10).

CelA is highly unusual in that it can hydrolyze both cellulose and hemicellulose in raw biomass to fermentable sugars, without the addition of other accessory proteins (see the figure, panel C). Brunecky *et al.* show that during biomass hydrolysis, fragments of CelA are released, allowing smaller enzyme fragments to reach regions of the substrate that the complete protein would not be able to access. The supplementation

Plant biomass is chemically complex. Cellulose is the main component of plant cell walls and constitutes 33% of all vegetable matter on Earth. Cellulose is a linear polysaccharide containing thousands of glucose units that form both amorphous and highly recalcitrant crystalline regions. The glucose units are linked to each other through very stable O-glycosidic bonds that are the targets of cellulases. Plant biomass also contains two other major polymers, hemicellulose and lignin, both of which are considered natural barriers

for cellulases. Unlike cellulose, hemicelluloses and lignin lack a recalcitrant crystalline structure and are very diverse.

For decades, the complementary action of cellulases (see the figure, panel A) and hemicellulases (panel B) in the enzymatic hydrolysis of cellulose and hemicellulose was the basis for the development of advanced industrial biomass enzymes (4). More recently, the discovery of other types of enzymatic synergies has aided the development of even more efficient industrial

of CelA with a β -glucosidase leads to complete hydrolysis of a very recalcitrant form of cellulose, microcrystalline cellulose, in a reasonable time frame (7 days). In addition, the high thermal stability of CelA would be seen by many in the biomass biorefinery industry as an advantage; operating a biomass biorefinery plant at high temperature dramatically reduces the chances of bacterial contamination and lowers the viscosity of the reaction mixes.

Most commercial biomass enzymes are of fungal origin, mainly because fungal enzymes can be produced in higher yields than enzymes from other sources. In addition to CelA, other bacterial enzymes can play an important role in enzymatic degradation of plant polysaccharides when combined with commercial fungal enzymes. For instance, Resch *et al.* (11) have

shown that a bacterial cellulosome preparation can substantially boost the hydrolytic activity of a fungal cellulase cocktail. Cellulosomes are multienzyme complexes of glycoside hydrolases anchored to noncatalytic subunits (scaffoldins); they are mainly found in bacteria, but fungal cellulosomes have also been reported.

Commercial efforts to make cellulosic liquid biofuels via enzymatic hydrolysis have come a long way since the late 1990s. But there is still room for improvement as new enzyme classes and new modes of action are discovered—such as the multifunctional cavity-forming CelA reported by Brunecky *et al.*—and optimally engineered enzymes are developed. Continued improvements should yield biomass enzymes that are even more robust and better adapted to the specific needs of the pro-

cesses deployed in the emerging global carbohydrate economy.

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GENOMICS

Genomic Clues to the Ancestral Flowering Plant

Keith Adams

Amborella trichopoda is an understory shrub that is endemic to New Caledonia (see the figure). It is an early-diverging flowering plant—most phylogenetic studies indicate that it diverged from the lineage leading to most flowering plants, and it is the single sister species to all other extant angiosperms (1). As such, it represents the equivalent of the duck-billed platypus in mammals (in the earliest branch of the mammalian family tree). Given its pivotal position in flowering plant phylogeny, it has been of considerable interest to sequence its genome. On page 1467 of this issue, the Amborella Genome Project (2) reports the nuclear genome sequence, with extensive analyses. In addition, on page 1468, Rice *et al.* (3) report the complete sequence of the Amborella mitochondrial genome, which contains a massive amount of horizontally transferred DNA. And on page 1516, Chamala *et al.* (4) describe a new assembly and validation approach for the Amborella nuclear genome that can be applied to other nonmodel eukaryotes.

The Amborella genome is the first nuclear genome sequence to be reported from the



The sequenced shrub. *A. trichopoda* is an early-diverging flowering plant. The sequence of its nuclear genome provides insights into plant evolution and clues to the genes present in the ancestral flowering plant.

basal angiosperms (early-diverging flowering plants). The complete genome has major implications for reconstructing features of an ancestral angiosperm genome. For example, the Amborella genome facilitates inference of the gene content of the earliest angiosperms. The genome project found that the ancestral angiosperm gene set contained at least 14,000 genes. Compared with nonan-

The genome sequence of *Amborella trichopoda* provides insights into the molecular evolution of flowering plants.

giosperms, 1179 gene lineages (orthogroups) first appeared in angiosperms (or have diverged sufficiently such that none of the gymnosperm homologs were detected), and about 4000 gene lineages are specific to seed plants. The new gene lineages in flowering plants may have led to gene functions specific to angiosperms and crucial for their diversification and success. New functions also were gained by preexisting genes (neofunctionalization), such as genes involved in flower development that have homologs in other seed plants. The study concludes that orthologs of most floral genes existed long before their specific roles were established in flowering, and they were later co-opted to serve floral functions.

Noncoding small RNAs play roles in floral and other developmental processes (among other functions). The Amborella genome contains at least 124 microRNA loci corresponding to 90 different families, with 27 of those families likely present in the ancestral angiosperm.

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Both protein-coding gene families and noncoding small RNA families are often analyzed with gene trees in molecular evolution research. Studies of gene families in any group of angiosperms can now use genes from *Amborella* as an outgroup (a reference for determining evolutionary relationships) in the analyses.

The *Amborella* genome provides clear structural evidence for an angiosperm-specific whole-genome duplication, which was previously inferred based on other data (5). It also provides additional evidence for the timing of that event at the branch leading to angiosperms in phylogenetic trees.

The *Amborella* genome sequence permitted reconstruction of the ancestral gene order in core eudicots by comparison to three eudicot genomes (grape, peach, and cacao). The hypothetical structure of seven inferred chromosomes in the ancestor of the core eudicots was reconstructed. Note that this was aided by the absence of an additional whole-genome duplication specific to the *Amborella* lineage. The ancestral eudicot genome reconstruction is useful for understanding how genomes have evolved in eudicots, which represent about 75% of angiosperm diversity. The ancestral eudicot genome reconstruction also will indicate how genome evolution has proceeded in different lineages after genome doubling (the gamma hexaploidy event) occurred early in eudicot evolution (6, 7).

Transposable elements in the *Amborella* genome are ancient, some of them having persisted for millions of years, but are no longer active. This is in contrast to many other angiosperm genomes in which transposable elements have proliferated, sometimes massively. The lack of transposable element activity in the *Amborella* genome may be due to very effective silencing mechanisms that prevent gene expression or the loss of active transposases that catalyze their movement (2); both could minimize or prevent their spread in the genome.

There are fewer than 15 known populations of *Amborella* in the world. Sequencing the genomes of 12 plants across this range revealed four genetically distinct clusters of populations and dynamic genome evolution. Despite its restricted geographic distribution, *Amborella* maintains substantial genetic diversity. Knowledge of genetic population structure may be helpful for guiding conservation efforts.

Large nuclear genomes from nonmodel eukaryotes are extremely challenging to assemble from relatively short sequencing reads derived from ultrahigh-through-

put sequencing (“next generation”) technologies. Chamala *et al.* used a new strategy to aid assembly (and its validation) that includes fluorescence in situ hybridization (FISH) to localize sequences by microscopy. Assembled chunks of the *Amborella* genome (scaffolds), representing about 68% of the assembled genome, were localized cytologically by FISH to assess their integrity and location on the chromosomes. The authors also used whole-genome mapping to help close gaps between scaffolds. This combined approach of shotgun sequencing, whole-genome mapping, and validation using FISH can be applied to other nonmodel organisms.

Rice *et al.* show that *Amborella* has one of the largest sequenced angiosperm mitochondrial genomes with five circular chromosomes. It contains a large amount of DNA that was transferred from other organisms, including mosses, flowering plants, and green algae. Some of the horizontally transferred genes were previously identified (8) but Rice *et al.* discovered and thoroughly characterized very large foreign regions including four whole-genome transfers. Horizontal transfer in *Amborella* may have been facilitated by its close association with other plants (epiphytes). Rice *et al.* propose a model whereby whole mitochondria were

captured and fused with the mitochondria of *Amborella*, after which genome recombination occurred. A low rate of mitochondrial DNA loss probably contributes to large horizontally transferred regions remaining in the genome for millions of years.

The sequencing, assembly, and analyses of the *Amborella* nuclear genome provide major insights into nuclear genome evolution in angiosperms and the kinds of genome features that were present in ancestral angiosperms. The information will be a useful resource for studies of genome evolution, gene family evolution, and phylogenetics.

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BIOCHEMISTRY

Enzyme Kinetics, Past and Present

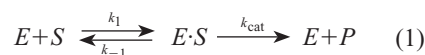
X. Sunney Xie

The Michaelis-Menten equation, first reported 100 years ago, holds at the single-molecule level.

Enzymes catalyze biochemical reactions, speeding up the conversion from substrate to product molecules. One hundred years ago, Leonor Michaelis and Maud Leonora Menten studied the equation characterizing enzymatic rates (1). This landmark development in the quantitative description of enzymes has stood the test of time, and the Michaelis-Menten equation remains the fundamental equation in enzyme kinetics (2). Today, the quest for fundamental understanding of the working of enzymes continues with vigor at the single-molecule level as new experiments and

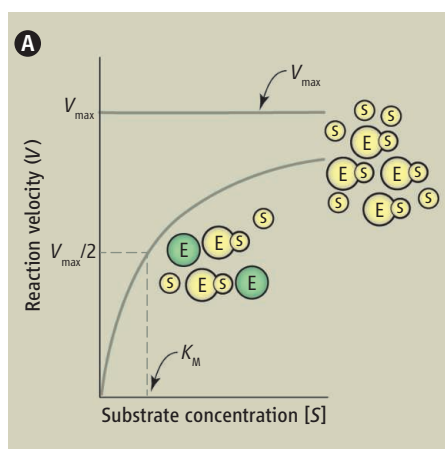
theories emerge.

Michaelis and Menten's theory is not about how an enzyme speeds up a reaction. Rather, it describes a kinetic scheme for the enzyme *E* and its substrate molecule *S* to form a complex before proceeding to the product *P*:

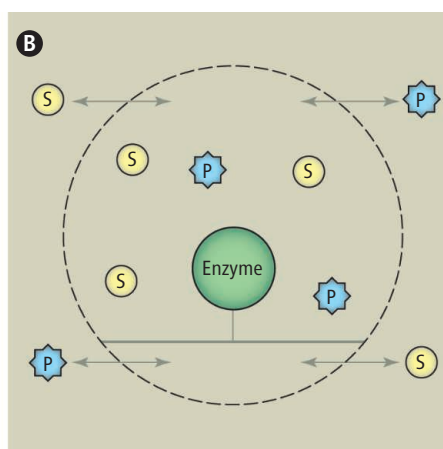


where k_1 and k_{-1} are the forward and reverse rate constants for substrate binding and k_{cat} is the catalytic rate constant. Assuming a fast equilibrium between *E* and *E*·*S*, they characterized the initial turnover rate of an enzyme, *V*, and derived the characteristic hyperbolic relationship between *V* and substrate concentration [*S*],

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Enzyme kinetics. (A) Enzyme reaction velocity as a function of substrate concentration according to the Michaelis-Menten equation. K_M is the concentration at which the enzymatic velocity reaches half of its saturation value, $V_{\max}$. (B) Nonequilibrium steady-state condition of a single enzyme turnover experiment. The substrate and product concentrations are held constant during the reaction catalyzed by the enzyme. A similar situation exists in live cells.



$$V = \frac{V_{\max}[S]}{[S] + K_M} \quad (2)$$

where $V_{\max} = k_{\text{cat}}[E]_T$ and $[E]_T$ is the total enzyme concentration. At low $[S]$, V is proportional to $[S]$, whereas at high $[S]$, V saturates (see the figure, panel A). K_M is the substrate concentration at which the velocity is half of the saturation level (1). A more general derivation of the equation was subsequently given by Briggs and Haldane (3, 4).

The Michaelis-Menten equation not only quantified the kinetics of enzymatic reactions but also provided a practical means for characterizing an enzyme in terms of k_{cat} and K_M . A high k_{cat} and a low K_M , or a high k_{cat}/K_M ratio, are indicators for an enzyme's effectiveness. In 1934, Lineweaver and Burk showed how to rearrange the Michaelis-Menten equation to facilitate determination of K_M and k_{cat} by plotting $1/V$ against $1/[S]$ (5). The work was extremely highly cited, but in the computer era the significance of this rearrangement is less profound.

Many reactions do not follow the simple scheme of Eq. 1, and thus the “apparent” k_{cat} and K_M are not so simple to interpret. A major experimental advance in enzyme kinetics came in the 1970s and 1980s, when stopped-flow and continuous-flow experiments allowed the process of reaching the steady state to be studied. Using rapid sample mixing and high time resolution monitoring, these techniques allowed many complicated enzyme kinetic schemes to be decoded and many enzymatic intermediates to be determined (6).

Another major experimental advance came in the 1990s, when single-molecule

enzymology was made possible by single-molecule fluorescence imaging at room temperature and single-molecule manipulation (7, 8). The time traces of such experiments cannot be repeated, but their statistical properties are reproducible (9); similar observations have been made for the electric signals from a single ion channel (10), the first single-molecule experiment in biology. Over the past two decades, single-molecule enzymology has provided insights into how specific enzymes—particularly molecular motors and nucleic acid enzymes—work at the molecular level.

Single-molecule studies have also led to many insights that are general to all enzymes. One such general phenomenon is dynamic disorder. The k_{cat} of a single enzyme molecule is not a constant but rather exhibits large fluctuations (9, 11). The fluctuation of k_{cat} results from the fact that an enzyme has a broad distribution of interconverting and long-lived conformational states (12), each of which has a different k_{cat} (11, 13). This fluctuation in k_{cat} remains hidden in conventional ensemble experiments.

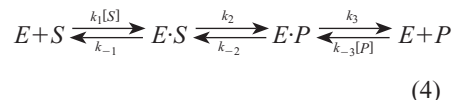
For the kinetic scheme in Eq. 1, single-molecule experiments have shown that the stochastic waiting time of an enzymatic reaction exhibits a distribution with an exponential rise followed by an exponential decay. For a single molecule with slowly interconverting conformational states with different k_{cat} and K_M (14), it follows that

$$\frac{1}{\langle \tau \rangle} = \frac{k_{\text{cat}}[S]}{[S] + K_M} \quad (3)$$

where $\langle \tau \rangle$ is the mean of the stochastic

waiting time and the overbars denote the weighted averages of k_{cat} and K_M of different conformational states. Comparison with Eq. 2 shows that the Michaelis-Menten equation holds at the single-molecule level despite the ubiquitous and large fluctuations in k_{cat} (13). This result has been verified experimentally (11). This underscores why the Michaelis-Menten equation works so well at the ensemble level.

In single-molecule turnover experiments, each enzyme experiences constant substrate and produce concentrations in the volume of interest (dashed circle). Each enzyme is thus effectively in a non-equilibrium steady-state condition, which mimics the situation inside a live cell. Furthermore, many reactions in cells are reversible. In this case, the kinetic scheme underlying the Michaelis-Menten equation needs to be modified to allow for such reversibility:



Hill has shown how the forward and backward reaction fluxes (J^+ and J^- , respectively) in a reversible enzymatic reaction can be related to the thermodynamic driving force (14, 15), namely the chemical potential difference, $\Delta\mu$, between product and substrate:

$$\Delta\mu = -kT \ln (J^+/J^-) \quad (5)$$

where k is the Boltzmann constant and T is the absolute temperature. Eq. 5 relates the enzymatic rate (that is, the difference between forward and backward fluxes) to chemical potential differences (which are determined by concentrations of reactants and products). In this sense, it is similar to the Michaelis-Menten equation, which relates enzymatic rates to substrate concentrations only. Equation 5 is more general than the Michaelis-Menten equation and is also more powerful, because it links the thermodynamic driving force to enzymatic kinetics. In the spirit of Michaelis and Menten, new experimental discoveries will no doubt lead to deeper understanding of enzyme kinetics and function, keeping scientists busy well into the next 100 years.

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10.1126/science.1248859

GEOCHEMISTRY

Reformulating Table Salt Under Pressure

Jordi Ibáñez Insa

Sodium chloride (NaCl), the main component of table salt, is the archetypical ionic compound of chemistry textbooks. The large electronegativity difference between the participating elements drives salt formation. Metallic sodium transfers electrons to chlorine, and the resulting positively and negatively charged ions are held together by electrostatic attraction—ionic bonds. At ambient conditions, NaCl crystallizes in the so-called rocksalt structure, a cubic array of Na and Cl atoms in equal proportions (1:1 stoichiometry) and with six-fold coordination. With increasing pressure, a structural phase transition to the cubic, eight-fold coordinated NaCl-B2 phase is observed at ~30 GPa [(1) and references therein]. Theory suggested that complete metallization of NaCl should occur above ~300 GPa, and no Na-Cl compounds other than NaCl were known to exist. However, on page 1502 of this issue, Zhang *et al.* (2) show that stable Na-Cl phases with stoichiometries different from 1:1 and intriguing properties can be synthesized in the lab with high-pressure techniques.

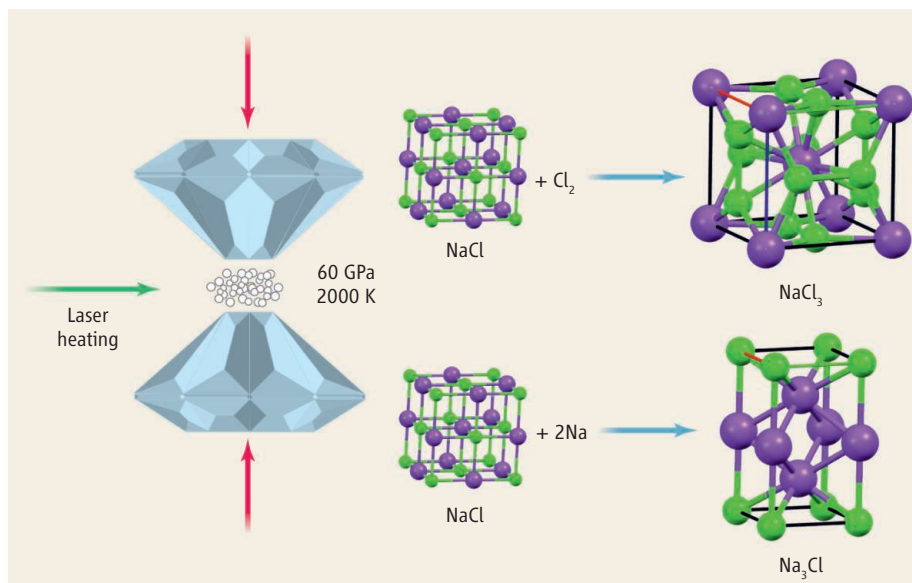
The application of high pressure allows researchers to dramatically change the bonding of atoms by reducing interatomic distances, and thus change the properties of solids. At sufficiently large pressures, insulators can become metals, soft chemical bonds become much stiffer, structural phase transitions take place, and chemical reactions that do not occur at ambient conditions become possible. By means of theoretical calculations with the ab initio evolutionary algo-

rithm USPEX (3), Zhang *et al.* predict that different Na-Cl compounds such as Na₃Cl, Na₂Cl, Na₃Cl₂, NaCl₃, or NaCl₂ are thermodynamically stable at nonambient pressure conditions. More important, they provide compelling experimental evidence that two of these compounds, most likely NaCl₃ and Na₃Cl, are formed by squeezing NaCl in Cl- or Na-rich conditions in a diamond anvil cell and by applying temperatures around 2000 K with laser heating (see the figure).

In hindsight, one might be tempted to claim that the observation of new stoichiometries in the Na-Cl system is not that surprising. The argument could be something like “Compress a highly ionic substance and

Sodium chloride transforms into exotic compounds such as NaCl₃ or Na₃Cl with compression, laser heating, and excess of Cl or Na.

you will end with covalent bonds and higher coordinations.” With increasing density upon compression, the inner electrons are expected to participate in the bonding process, making covalent bonds energetically more favorable. However, these new Na-Cl phases remain stable down to relatively low pressures (20 GPa for NaCl₃). In addition, these phases exhibit remarkable crystal structures, chemical bonding, and electronic properties. Finally, the experimental confirmation of the theoretically predicted Na-Cl phases adds another success for USPEX, which shows an excellent ability to predict the stability of yet unknown compounds. The present results will surely stimulate both theoretical and experimental investi-



Reformulating table salt. When sodium chloride (NaCl) is squeezed by diamond anvils at high temperatures and under Cl- or Na-rich conditions, compounds such as NaCl₃ or Na₃Cl can be formed. Zhang *et al.* theoretically predicted the stability of these phases and confirmed this prediction experimentally with x-ray diffraction and Raman-scattering measurements.

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gations to produce high-pressure phases with unusual stoichiometries and potentially new, exotic properties.

Still, the most intriguing aspect of this work is that it represents the fall of a textbook idol. Under high pressure, the familiar rules of chemistry are modified and the simplicity of highly ionic compounds such as NaCl is totally lost. If NaCl₃, Na₃Cl, and other such chemical formulae are possible, one must wonder about the stability under extreme conditions of a plethora of new phases with modified stoichiometries of other “more familiar” compounds. Such compounds could have particularly important implications in geological and planetary sciences, as most of the matter in stars and planets, including Earth, is subject to very high pressures and temperatures.

There already exist some examples of exotic compounds that could be formed in planetary interiors, including a new magnesium-iron (Mg-Fe) carbon-bearing phase with a structure based on (CO₄)⁴⁻ tetrahedra (4). This compound, obtained at pressure and temperature conditions equivalent to depths of 1800 km in Earth, could have relevance to the global carbon cycle. The noble gas xenon (Xe) can react with ice at high pressures and

temperatures (5). The resulting Xe-bearing phases could explain the observed depletion of Xe in the atmospheres of giant planets such as Uranus and Neptune. Also, unexpected stoichiometries of Mg-O compounds have already been predicted to be thermodynamically stable at pressure and temperature conditions of some giant planets (6).

In 2004, it was shown that the perovskite MgSiO₃, which is thought to be the major constituent of Earth's lower mantle, undergoes a phase transition to a postperovskite structure above 120 GPa and 2500 K (7, 8). This phase seems to partly explain the anomalous behavior of seismic waves in Earth's lower-mantle region known as the *D''* layer, near the core-mantle boundary (9). However, there are still unresolved issues with regard to the characteristics of the *D''* region and the role played by postperovskite phases (10, 11). The existence of a single (stable) exotic compound at such depths would open up a surprising new avenue in geophysics and Earth dynamics research. Could the lower mantle, and particularly the *D''* layer, contain appreciable amounts of one or more unexpected unknown phases of the Mg-Fe-Ca-Si-Al-O system? Could (Mg,Fe)SiO₃ or any of these exotic compounds incorpo-

rate, under the extreme conditions of the *D''* region, sizable concentrations of atoms (K, Na, C, N, etc.) that are not soluble in the perovskitic phases of the lower mantle? And what about the planetary interiors of giant planets with regard to the C-H-N-O systems and noble gases such as He and Ne? The answers to these provocative questions might completely modify our understanding of the physics and chemistry of a large fraction of matter in our solar system and beyond.

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CELL BIOLOGY

Ronning After the Adiponectin Receptors

William L. Holland¹ and Philipp E. Scherer^{1,2}

Since its initial description in 1995 (1), few molecules in metabolism research have received as much continued interest as adiponectin. The focus of more than 12,000 publications, this adipokine has been widely studied in preclinical and clinical settings under a variety of physiological and pathophysiological conditions (2). Adiponectin is produced by adipocytes and released into circulation. It is considered protective, based on the potent insulin-sensitizing, antilipotoxic, anti-apoptotic, and anti-inflammatory actions it exerts on different cell types. These compelling effects marked adiponectin

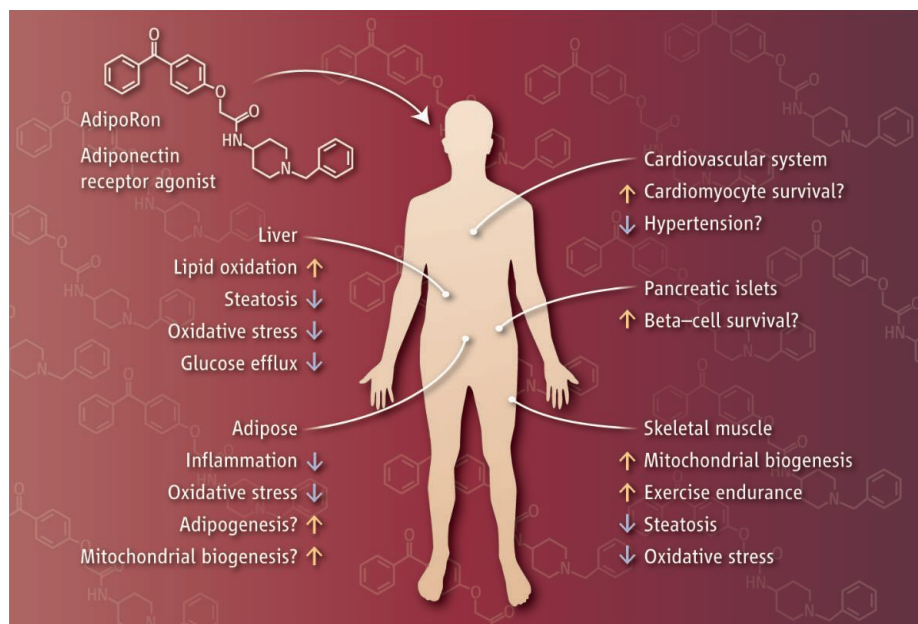
as a possible drug target for diabetes and other obesity-associated diseases. The long wait for a small-molecule agonist for adiponectin receptors may soon be over. Okada-Iwabu *et al.* (3) have identified a compound that is an adiponectin receptor agonist in rodent and cell culture models. It represents an important step toward filling an unmet clinical need for additional therapeutic options against diabetes, obesity, and other associated disorders.

Unlike the vast majority of other adipocyte-derived factors, adiponectin enjoys a reputation of being a “friendly” adipokine whose circulating concentration increases under metabolically favorable conditions and decreases under conditions of obesity-induced metabolic stress (as compared to other adipokines, adiponectin secretion is unusual; the more adipose tissue one has,

A small-molecule drug mimics the beneficial effects of adiponectin in cells and in animal models of diabetes and obesity.

the less adiponectin is found in circulation). Its actions on hepatocytes, endothelial cells, pancreatic β cells, and cardiac myocytes have been reported in rodent studies and are substantiated by clinical correlations. Indeed, mice that constitutively overexpress adiponectin are protected against metabolic challenges, including those imposed by a high-fat diet. A modest amount of adiponectin also conquered the genetic challenge of the *ob/ob* mouse, rescuing its diabetic phenotype (4). Adiponectin improved survival in mouse models of cell-type-specific apoptosis as well (5). Not only have genetic gain-of-function mutations in animals demonstrated the potent actions of this protein, but adiponectin produced in vitro from recombinant DNA can induce responses in animals that are comparable to those elicited by genetic overexpression of the adipokine (6, 7).

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Adiponectin mimetic. Muscle, heart, pancreas, liver, and adipocytes are key target tissues for adiponectin receptor activation. The small molecule AdipoRon has the potential to induce insulin sensitivity, as well as anti-inflammatory and anti-apoptotic effects, in a wide range of tissues due to the widespread expression of adiponectin receptors.

Many of the cellular effects of adiponectin became better understood when the receptors for adiponectin, AdipoR1 and AdipoR2, were identified in 2003 (8). This spurred efforts to produce recombinant bioactive adiponectin (9). Although these preparations were active and insulin sensitizing, several issues made its large-scale production challenging. Adiponectin is difficult to produce in its full-length form in bacteria. It also requires several posttranslational modifications in its collagenous amino terminus, which can only be achieved if produced in mammalian cells. It is also a homo-oligomer that assembles into higher-order structures that consist of trimers, hexamers, and high molecular weight species of 12 to 36 oligomers that circulate in plasma as large complexes of ~800 kD (10). And although adiponectin circulates at microgram per milliliter concentrations in plasma, it turns over quickly, with a half-life of 45 to 60 min in the mouse (11). The complex quaternary structure and rapid turnover are major disadvantages to producing and administering adiponectin in amounts that can be sustained over time and in a cost-effective manner. Thus, the field has been awaiting the advent of low molecular weight agonists for adiponectin receptors that would overcome production bottlenecks.

Okada-Iwabu *et al.* screened a compound library and identified several molecules that activate adiponectin receptors but focused their in-depth analysis on one, “AdipoRon.”

AdipoRon binds, at a low micromolar concentration, to both AdipoR1 and AdipoR2. Like adiponectin, it activates 5'-adenosine monophosphate-activated protein kinase (AMPK) in cultured mammalian cells, an enzyme that is involved in many metabolic processes including the release of insulin, inhibition of lipid synthesis, and stimulation of glucose uptake. It also activates the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC1 α), which boosts mitochondrial proliferation and energy metabolism. Like adiponectin, AdipoRon improved glucose metabolism, lipid metabolism, and insulin sensitivity in cultured cells and in mice by mechanisms requiring the presence of adiponectin receptors. When *db/db* mice (an animal model for type II diabetes and obesity) fed a high-fat diet were treated with AdipoRon (by oral administration), the metabolic improvements also extended their life span. Furthermore, the authors demonstrated that giving chow-fed wild-type mice AdipoRon enhanced their exercise endurance capacity. The study makes a convincing case that targeting adiponectin receptors with low molecular weight agonists is a viable strategy, and that developing higher-affinity agonists with improved pharmacokinetics should be pursued.

Adiponectin receptor activation is a promising potential therapy for diabetes, nonalcoholic fatty liver disease, cardiovascular disease, anti-inflammatory action in

macrophages, and cytoprotective effects on pancreatic β cells (see the figure). Moreover, AdipoRon is orally administered and readily absorbed and delivered to relevant target tissues, making the approach even more attractive (some compounds are highly partitioned into certain tissues, are poorly absorbed, or are too rapidly altered to be of therapeutic benefit). The next steps should include evaluating whether AdipoRon stimulates a ceramidase activity in cells (thereby decreasing the amount of the sphingolipid ceramide), the most proximal cellular response to receptor activation (5, 12). There are also several concerns that must be addressed. Notably, mice genetically engineered to overexpress adiponectin show reduced bone density. This is also true of molecules that stimulate adiponectin secretion in rodents (such as thiazolidinediones and fibroblast growth factor 21) (13, 14). Furthermore, heart damage (left ventricular hypertrophy) has been observed in rodents upon chronic increase of adiponectin production. Lastly, adiponectin may promote adipogenesis and angiogenesis associated with weight gain and growth of established tumors, respectively (7). Chronically elevated adiponectin concentrations can trigger infertility (15). These are potential side effects that will need to be scrutinized in great detail.

Despite these concerns, AdipoRon does not promote weight gain in mice, and the reported observation of a prolonged life span in *db/db* mice helps obviate some of these concerns, even though many of these effects require a more chronic exposure to be manifest. Nevertheless, the weight-neutral improvements in insulin sensitivity evoked by this adiponectin mimetic represent an important proof-of-principle and pave the way for developing much needed therapies.

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DNA Barcoding from NYC to Belize

Stephen E. Harris^{1,2†} and Marissa Bellino^{2,3}

The Student DNA Barcoding Project, an IBI prize-winning module, develops student-generated research to study local biodiversity using molecular biology skills.

Traditional means of studying biodiversity depend on expert knowledge from individuals with years of education and training. Recent techniques like DNA barcoding, the process of identifying species based on short fragments of DNA, can be used to quickly identify species and to provide easy access to taxonomic information, a particular benefit to both students and developing nations

(1). We use DNA barcoding as the foundation for introducing students to modern biological research. Initially, we set out to develop a research course that serves as an alternative to more traditional laboratories, which often have known outcomes and lack student-generated investigations. Our goal was to provide an experience and skill set to students that would drive interest in the sciences and prepare them for the rigors of studying Science, Technology, Engineering, and Mathematics (STEM). Although we are in the age of genomics, too often the practical knowledge and necessary skills to succeed in science are not taught to students at the high school or even undergraduate level.

The Student DNA Barcoding Project curriculum allows students to pursue research projects by using field ecology and advanced molecular biology methods. Students are exposed to possible careers in STEM fields and the laboratory methods serve as the foundation for future research in academic labs. The course materials outline the infrastructure needed for a teacher to run his or her own DNA barcoding lab at a high school or undergraduate institution, arguably the biggest obstacle in pursuing molecular biology research. Plans for low-budget portable lab space and larger, more



NYC students working with curriculum. (A) Students cataloging diversity and collecting samples from Inwood Hill Park in Northern Manhattan for unit 1 of the curriculum. (B) Students in the science research lab at their high school, extracting DNA in order to generate barcodes.



advanced labs are included, and all protocols use nontoxic reagents. The course has been field-tested in New York City (NYC) and Belize, and the resulting DNA sequences, made publicly available on The Barcode of Life Data Systems, are a resource for the international scientific community.

The Student DNA Barcoding Project began in 2010 at a public high school in NYC through the National Science Foundation's (NSF's) Graduate STEM Fellows in K–12 (kindergarten through high school) Education (GK–12) program conducted by the Center for Advanced Study in Education at the Graduate Center. Within the science research class, we realized that there was an opportunity to integrate laboratory training, mentorship, and student-generated research (see the photos). We decided to seek funding to build a molecular lab space in our school, a place where students and scientists could meet and learn from one another. A companion curriculum was developed to introduce ecological and molecular knowledge and skills with a focus on local and authentic research questions.

The yearlong curriculum has five major units designed to culminate with student-developed research questions; however, it is flexible enough that educators can work with individual units to fit time restraints and specific student populations. Reading scientific literature is embedded throughout the curriculum and students are introduced to the C.R.E.A.T.E. protocol (2), a method for unpacking complex scientific text and generating research questions.

In unit 1, Sampling Local Biodiversity, students practice observation, questioning,

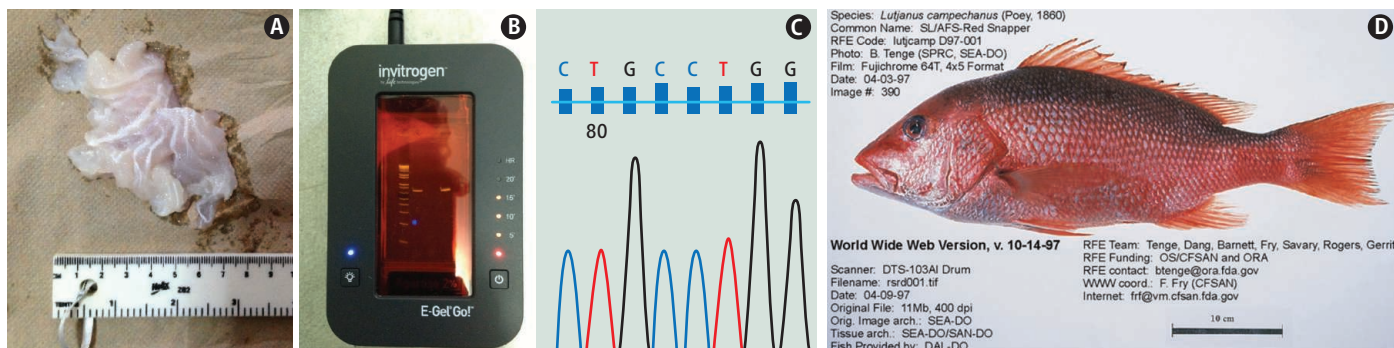
and ecological research methods by collecting local samples as a first step in generating a DNA barcode. Emphasis is placed on systematically documenting information about samples. Sampling can occur in local parks as a way to inventory biological diversity or at local markets to investigate potential mislabeling.

Unit 2, Molecular Biology: Theory and Practice, introduces students to the protocols and laboratory skills needed to produce and analyze DNA barcodes. Students learn to efficiently manage small volumes with micropipettes and are introduced to DNA extraction, amplification via the polymerase chain reaction, and gel electrophoresis.

Units 3 and 4, The Science of DNA Barcoding and Analyzing DNA Barcodes, respectively, both build on processing samples from Unit 1. In the lab, students sequence the *cytochrome oxidase 1* gene to generate a DNA barcode for their sample and move to the classroom where they use bioinformatics to turn a string of nucleotides into information that can be used to answer biological questions. They are trained to process DNA by checking the overall quality, manually editing and trimming low-quality bases, understanding and using tools like BLAST (3) to identify the species of unknown samples, and aligning sequences across multiple species in order to investigate evolutionary relationships. We take advantage of the user-friendly bioinformatics pipeline implemented in DNA Subway (4) to teach analysis skills and later use the Barcode of Life Data System Student Data Portal (BOLD-SDP) (5) to

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*IBI, Science Prize for Inquiry-Based Instruction; www.sciencemag.org/site/feature/data/prizes/inquiry/.



Belizean investigation of mislabeled local fish. (A) Size and physical characteristics of purchased fish fillets. (B) Results from gel electrophoresis confirming amplification of *COI* gene. (C) Trace file of DNA sequence from local fish fillet sample. (D) Red Snapper, confirmed by DNA barcode but mislabeled by vendors.

create a permanent resource for student-generated barcodes.

In unit 5, Generating DNA Barcoding Research Questions, students develop proposals and produce results that can be presented at local science competitions, or uploaded online to the GenBank Sequence Database or the Barcode of Life Data System. Example research projects include finding the genetic diversity of bed bugs in NYC identifying bioindicator species in polluted parks, and investigating the mislabeling of fish fillets from local fish markets in Belize.

We tested the versatility of the curriculum by implementing different units outside of NYC. In 2012, we partnered with The Petters Research Institute in Dangriga, Belize (www.pribelize.org), where we adapted unit 1, Sampling Local Biodiversity, into a weeklong workshop for 24 local students, ages 12 to 16. We introduced concepts like biodiversity, sampling for scientific study, and the value of conserving local

ecosystems. We took students into a nearby empty lot and had them collect insects, taught them how to mount and identify the samples to the order level, and showed students how to build a Web site to share information with the public.

Similarly, we adapted units 3 and 4 for an intensive 3-day DNA barcoding workshop at Galen University in San Ignacio, Belize. We worked with undergraduate and master's students to investigate fish sold in local markets because of a previous report of fish mislabeling in Belize (6) (see the images). Students successfully sequenced 9 out of 12 samples and found that 66% of fish fillets were mislabeled. Pictures, global positioning system (GPS) coordinates, and DNA sequences are publicly available on the BioBelize Web site, and students presented the project and results during a nationwide evening news broadcast. Given our success over the last 2 years, we established The Biodiversity Center of Belize to continue our work (BioBelize, www.biobelize.org).

BioBelize uses a locally relevant curriculum in order to engage Belizean students in STEM fields.

To date, more than 150 students from NYC and Belize have participated in parts of our DNA Barcoding course. U.S. students engaged in the curriculum have competed in multiple science competitions with one student receiving a scholarship from Cornell University to continue her research from unit 5. In Belize, student projects are publicized online, and DNA results are stored in the Barcode of Life Data System Student Data Portal. Our DNA Barcoding curriculum is suitable for both high school science classes and university courses. The course is modular and transportable, and it guides educational facilities in the creation of a functional lab space where student researchers can study local biodiversity.

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Supplementary Materials

www.sciencemag.org/content/342/6165/1462/suppl/DC1

About the authors



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Research Fellow. **Marissa Bellino** is a New York City high school teacher and director of Education and Outreach at BioBelize. She runs the science research program at her school and built a molecular research laboratory for students to investigate urban ecology in New York City. Marissa was the recipient of the 2011 Sloan Award for Excellence in Teaching Science and Mathematics and is currently enrolled in the Urban Education Ph.D. program at CUNY–The Graduate Center.



SCIENCE JOURNALISM

Science Communication Requires Time, Trust, and Twitter

News Editor Robin Lloyd's "daily diet" of information includes more than 200 e-mails, thousands of tweets, and 70 or more stories in progress at *Scientific American*. With this schedule, she is not too happy when she receives press pitches that are not targeted to her publication.

Journalists and public information officers (PIOs) need strong social media savvy in the era of 24-hour news cycles, but positive working relationships and an eye for

"Twitter has become basically another major newspaper for me," said Lloyd. "A lot of science writers are on it, so it's a really great place to develop relationships and see what's going on in our field. Don't let anyone dismiss Twitter to you for that reason. For our goal as science communicators, it's massively important."

Panel moderator Robert Lee Hotz, science writer for *The Wall Street Journal*, pointed out that social media offer "a spec-

Inundated by new reports and story possibilities every day, science journalists value each of those minutes. Elizabeth Landau, a writer and producer for CNN.com, urged PIOs to learn more about reporters' interests by checking the Twitter lists they follow. "This is a way for you to see what 'beats' journalists find important," she explained. "Please don't call me. We just don't have the time."

Landau also stressed that images and video go a long way toward selling a science story. "It's almost as important as the headline, sometimes even more so," she noted, saying that a good photo can sometimes win coverage for one story over another.

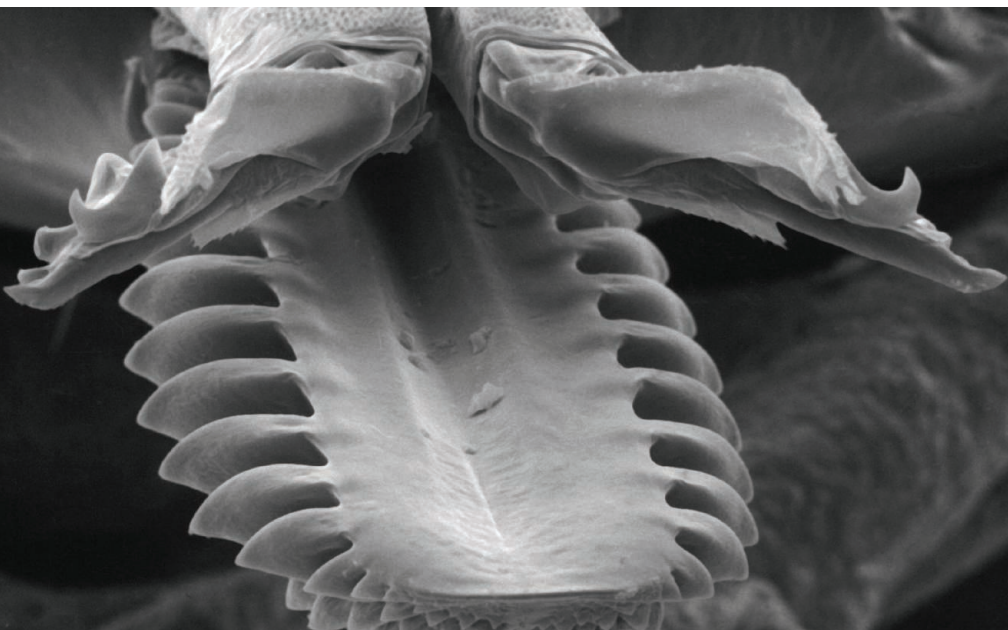
Today, researchers routinely capture video of animal behavior or brain cells firing, or they create animations to illustrate key phenomena, said James Gorman, a science reporter for *The New York Times*. All of those materials can be turned into compelling videos that avoid boring "talking head" interviews and might help to raise public awareness of the importance of the work.

"Cute videos work well, but gruesome videos work even better," Gorman said, eliciting squirms from attendees with a one-minute video of a wood tick chewing into the ear of a hairless mouse.

Playful videos and tantalizing tweets aside, the panelists agreed that delivering solid news from trustworthy sources and building personal relationships at venues like the AAAS Annual Meeting are still important for PIOs. Journalists and PIOs are increasingly working together to create stories on social media, said Brandon Keim, a freelance journalist at Wired.com. "The journalist isn't necessarily the center of information distribution anymore. It's much more egalitarian than that."

As science communication becomes more social and speedy, there will be more pressure on PIOs to get the facts straight and to avoid hyperbole, Hotz said. "You are starting to have to follow some of the same kinds of journalistic values that we do because your Twitter message may get directly retweeted without any intervening filter, and if you make things up, that becomes news—that becomes entertaining. And if you exaggerate, all in a good cause, of course, you can find yourself in the middle of a very big, boiling pot of Twitter-hashtag soup."

—Ginger Pinholster and Becky Ham



A penetrating image. A recent study about how ticks burrow into skin using a mouthpart called a hypostome offered ample opportunity to capture the public's attention with striking visuals, according to James Gorman, who covered the story for *The New York Times*.

real news value remain essential, Lloyd and others said at a recent National Press Club seminar organized by EurekAlert!, the science-news service of AAAS.

At the seminar, "Communicating Science Across Online and Social Media," 175 PIOs and other attendees from across the country listened to a panel of top journalists describe the changing ecosystem for science communication, particularly on Facebook, Twitter, Reddit, and other social platforms.

tacular opportunity" for anyone to communicate science news broadly. In 2012, for example, the landing of NASA's Curiosity rover on Mars generated 1.2 billion Twitter messages, 17.4 million Facebook hits, and 36.4 million webcast streams. During the actual seven-minute landing, 3.2 million viewers watched the event on UStream TV. The mission's Web site received 127 million page views, and the Twitter message announcing the landing was retweeted 72,000 times. "That's not bad for seven minutes' work," Hotz quipped.

HUMAN RIGHTS

On-Call Scientists Celebrate 5 Years

When Human Rights Watch asked Keith B. Ward to assist with a major report about the Ghouta chemical attack in Syria this August, the retired government scientist began analyzing the grim images and medical reports coming out of that country to confirm that chemical weapons had been used.

“Viewing, and actually having to study in some detail, the large number of photos and videos of suffering, dying, and dead victims was indeed distressing,” Ward said. “But my previous training with the Department of Defense, the Department of Homeland Security, and the FBI had prepared me to focus on the task at hand even in the face of emotionally charged situations.”

Ward is one of nearly 800 scientists, engineers, and health professionals who have volunteered their time and expertise to the On-call Scientists program, launched in October 2008 by AAAS’s Scientific Responsibility, Human Rights and Law Program. On-call volunteers work with human rights organizations on a variety of projects, from documenting discrimination and torture to studying the impacts of child labor and environmental degradation.

At a 5 December reception held at AAAS headquarters, Senior Program Associate Theresa Harris joined others in celebrating International Human Rights Day with the debut of a video series about the On-call partnerships.

The series helps to highlight the critical needs of human rights organizations that tackle scientific issues but may lack dedicated scientific staff. Five years after the program’s launch, “there is now a depth and breadth of experience that allows us to build multidisciplinary teams and advisory panels for human rights organizations, in addition to the referrals for one-time projects,” said Harris.

On-call volunteer Georgene Mortimer, an expert in the investigation and remediation of petroleum-contaminated sites, is assisting the Environmental Defender Law Center (EDLC) with two lawsuits in Africa and North America against major oil companies. Her technical analyses of pollution and its potential health impacts have been invaluable to the EDLC’s work, said the organization’s director, Lewis Gordon.

“When I can say to groups in developing countries, ‘I’ve got a pro bono scientist for you with years and years of training and experience,’ it’s like a gift from heaven,” Gordon noted.

Mortimer found the program when she was searching for a way to get involved with the human rights community, but she said others might choose to join “just to feel the personal satisfaction of having your work improve the lives of others.”

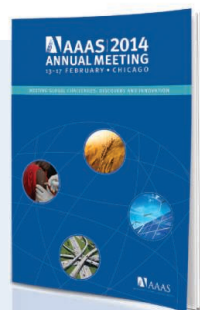
The impact of these volunteers can be immediate and rewarding, agreed Peter Bouckaert, Human Rights Watch’s director of emergencies. Bouckaert worked with Ward on the Ghouta report.

“Keith’s collaboration with Human Rights Watch resulted in a groundbreaking report on Syria’s chemical weapon use and ultimately helped put in motion Syria’s accession to the Chemical Weapons Convention,” he said. “It is a collaboration that helped save countless lives—what more satisfaction could any scientist wish for in a collaboration?”

—Becky Ham

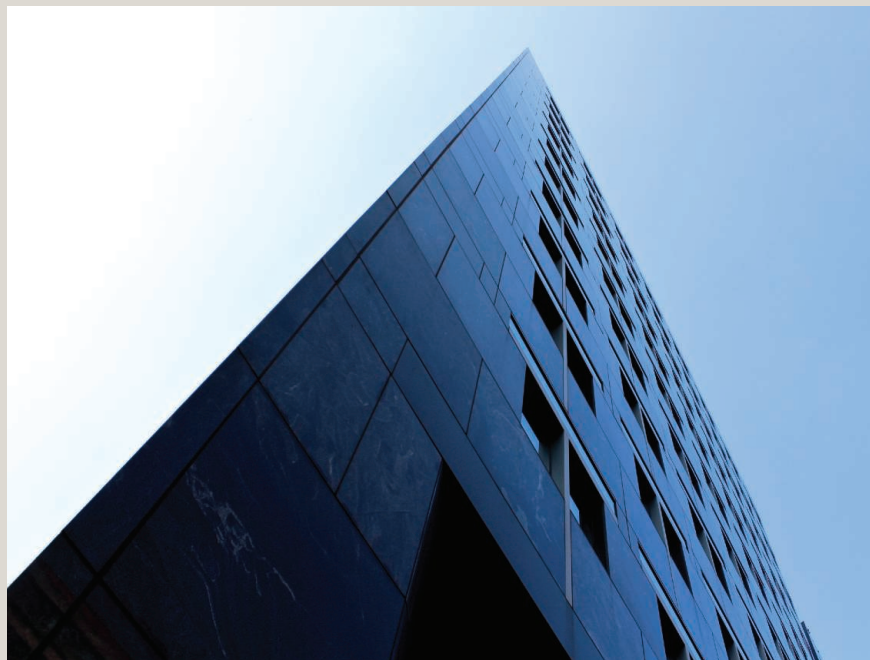
AAAS 2014 Annual Meeting

Investments in science and technology are major drivers of economic growth in many countries, making them a crucial piece of the recovering global economy. In February 2014, thousands of scientists, journalists, students, and others will convene in Chicago to share innovative ideas that encourage economic growth and offer solutions to the world’s challenges in food, fuel, climate, health care, and much more. Visit the 2014 Annual Meeting Web site at <http://meetings.aaas.org> to see the full program of scientific symposia, lectures, career workshops, and free public events.



ASSOCIATION AFFAIRS

AAAS Building Earns LEED Platinum Rating



AAAS has earned one of the highest marks awarded to existing green buildings: a LEED Platinum EB rating from the U.S. Green Building Council’s Leadership in Energy and Environmental Design (LEED) program. The rating system provides an internationally recognized, independent verification of a building’s performance with regard to energy savings, water efficiency, carbon emission reduction, and other measures of environmental impact. The AAAS building is one of the few previously existing structures in Washington, D.C., to earn the platinum rating.

PEPFAR: A Triumph of Medical Diplomacy

Many Americans are unfamiliar with the U.S. President's Emergency Plan for AIDS Relief (PEPFAR) and would be surprised to learn how successful it has been, according to Harold Varmus, director of the National Cancer Institute. Writing in the AAAS quarterly *Science & Diplomacy*, Varmus noted that the program had supplied an estimated 5 million patients in the developing world with antiretroviral drugs by 2012—up from 1.7 million in 2008—while protecting nearly 1 million infants from maternally transmitted HIV and testing nearly 50 million people for infection.

The numbers are only part of the story, however. By achieving solid bilateral partnerships with African countries, PEPFAR built and strengthened U.S. ties in that region, Varmus wrote in his 1 December essay.

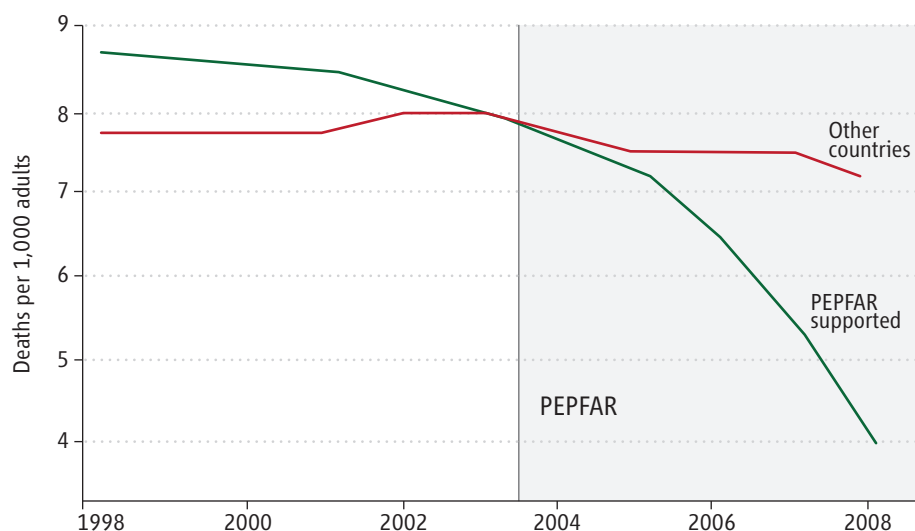
Those who aren't aware of PEPFAR's success would likely be further "astounded to learn that it was invented, in a remarkably direct way, by President George W. Bush, whose reputation in international affairs is dominated by his war on terrorism, military interventions in Iraq and Afghanistan, and the antagonism he displayed to the United Nations and to several of our traditional partners," according to Varmus.

In fact, President Bush was deeply

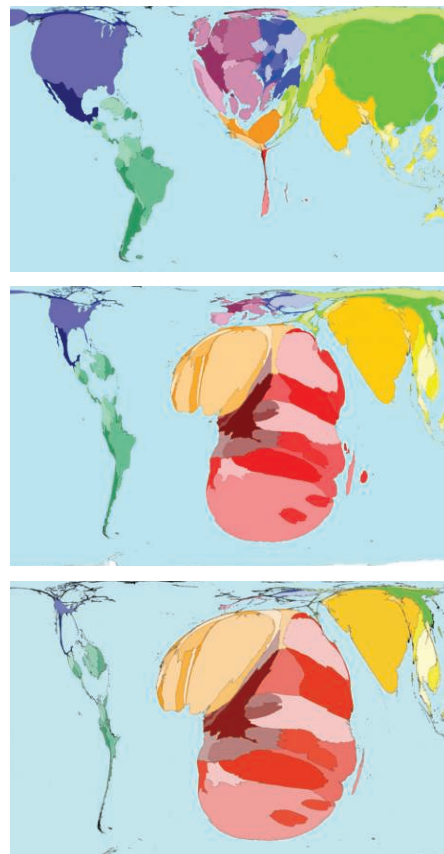
involved strategically at every stage of the project. Drawing from interviews of people who were critical to PEPFAR's planning and implementation, Varmus concluded that much of the program's effectiveness was due to its founders' emphasis on careful planning, bilateral relationships, objectives that were measurable and likely to be achieved, mandated evaluations, and advice from appropriate experts. Such advice proved critical in response to initial concerns that the \$15 billion project, which was planned "under a blanket of strict secrecy" to avoid interagency strife, would be difficult to finance and impossible to achieve, thus exposing Bush to criticism and failure.

The president's staff therefore asked Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, who along with his colleagues at NIAID had created the blueprints for PEPFAR, to invite several prominent physicians to speak with skeptics and critics from the Office of Management and Budget and other parts of the White House. The four invited physicians sensed the importance of the request and assembled rapidly from as far away as Rwanda and Uganda upon receiving Fauci's call.

The evidence from these experts who had worked in the field was crucial to convincing



Progress and disparity. Death rates in PEPFAR-supported African countries have fallen since the program's inception. The AIDS epidemic in Africa is far from over, however. Maps reflecting the global distribution of working physicians (top), HIV infections (middle), and AIDS-related deaths (bottom) in 2002 to 2004 show the disease's outsized burden compared to available medical care.



the president's representatives that the program should go forward, a decision reached just in time to be announced in President Bush's 2003 State of the Union address. Funding was secured soon after, and the program went on to improve life expectancy substantially in Africa.

"The PEPFAR history that Varmus has pieced together shows the power of science and medicine in diplomacy when they are used to promote humanitarian goals, even when projects are extremely complex and ambitious," said Vaughan Turekian, who is *Science & Diplomacy's* editor-in-chief and the director of AAAS's Center for Science Diplomacy.

Science & Diplomacy's December edition closes a second successful year for the online publication, which features a mix of perspectives and research articles by science and diplomacy practitioners and thinkers. The quarterly has published over 40 articles, on topics such as national approaches to science diplomacy, international research infrastructures, relationship building, and transboundary issues.

The *Amborella* Genome and the Evolution of Flowering Plants

Amborella Genome Project*†

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<http://dx.doi.org/10.1126/science.1241089>



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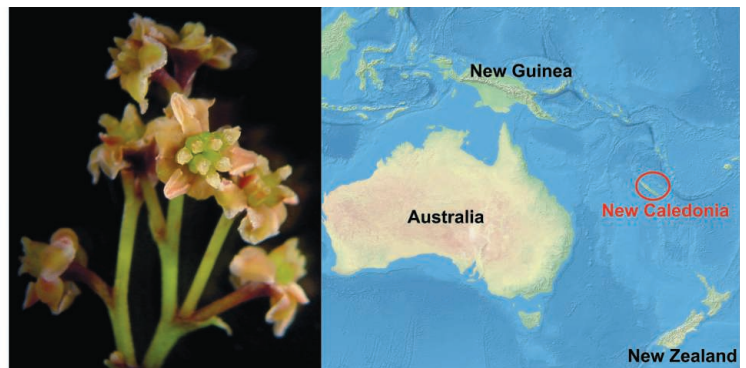
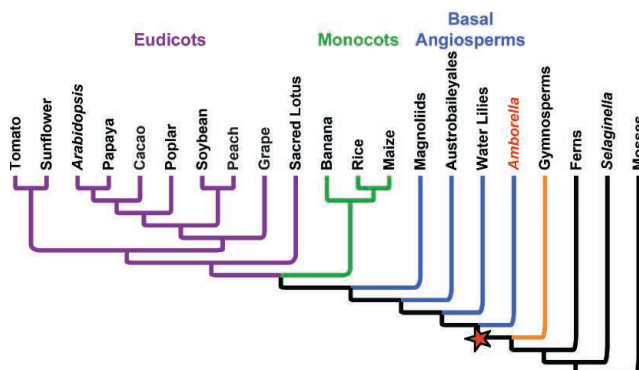
Introduction: Darwin famously characterized the rapid rise and early diversification of flowering plants (angiosperms) in the fossil record as an “abominable mystery.” Identifying genomic changes that accompanied the origin of angiosperms is key to unraveling the molecular basis of biological innovations that contributed to their geologically near-instantaneous rise to ecological dominance.

Methods: We provide a draft genome for *Amborella trichopoda*, the single living representative of the sister lineage to all other extant flowering plants and use phylogenomic and comparative genomic analyses to elucidate ancestral gene content and genome structure in the most recent common ancestor of all living angiosperms.

Results: We reveal that an ancient genome duplication predated angiosperm diversification. However, unlike all other sequenced angiosperm genomes, the *Amborella* genome shows no evidence of more recent, lineage-specific genome duplications, making *Amborella* particularly well suited to help interpret genomic changes after polyploidy in other angiosperms. The remarkable conservation of gene order (synteny) among the genomes of *Amborella* and other angiosperms has enabled reconstruction of the ancestral gene arrangement in eudicots (~75% of all angiosperms). An ancestral angiosperm gene set was inferred to contain at least 14,000 protein-coding genes; subsequent changes in gene content and genome structure across disparate flowering plant lineages are associated with the evolution of important crops and model species. Relative to nonangiosperm seed plants, 1179 gene lineages first appeared in association with the origin of the angiosperms. These include genes important in flowering, wood formation, and responses to environmental stress. Unlike other angiosperms, the *Amborella* genome lacks evidence for recent transposon insertions while retaining ancient and divergent transposons. The genome harbors an abundance of atypical lineage-specific 24-nucleotide microRNAs, with at least 27 regulatory microRNA families inferred to have been present in the ancestral angiosperm. Population genomic analysis of 12 individuals from across the small native range of *Amborella* in New Caledonia reveals geographic structure with conservation implications, as well as both a recent genetic bottleneck and high levels of genome diversity.

Discussion: The *Amborella* genome is a pivotal reference for understanding genome and gene family evolution throughout angiosperm history. Genome structure and phylogenomic analyses indicate that the ancestral angiosperm was a polyploid with a large constellation of both novel and ancient genes that survived to play key roles in angiosperm biology.

***Amborella trichopoda*, an understory shrub endemic to New Caledonia, is the sole surviving sister species of all other living flowering plants (angiosperms).** The *Amborella* genome provides an exceptional reference for inferring features of the first flowering plants and identifies an ancient angiosperm-wide whole-genome duplication (red star). *Amborella* flowers have spirally arranged tepals, unfused carpels (female; shown), and laminar stamens.



FIGURES IN THE FULL ARTICLE

Fig. 1. *Amborella* is sister to all other extant angiosperms.

Fig. 2. Synteny analysis of *Amborella*.

Fig. 3. Ancestral reconstruction of gene family content in land plants.

Fig. 4. *Amborella* as the reference for understanding the molecular developmental genetics of flower evolution.

Fig. 5. Classification and insertion dates of LTR transposons in the *Amborella* genome.

Fig. 6. Population genomic diversity in *Amborella*.

SUPPLEMENTARY MATERIALS

Supplementary Text

Figs. S1 to S42

Tables S1 to S46

Additional Acknowledgment

References

RELATED ITEMS IN SCIENCE

K. Adams, Genomic clues to the ancestral flowering Plant. *Science* **342**, 1456–1457 (2013).
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The *Amborella* Genome and the Evolution of Flowering Plants

Amborella Genome Project*†

Amborella trichopoda is strongly supported as the single living species of the sister lineage to all other extant flowering plants, providing a unique reference for inferring the genome content and structure of the most recent common ancestor (MRCA) of living angiosperms. Sequencing the *Amborella* genome, we identified an ancient genome duplication predating angiosperm diversification, without evidence of subsequent, lineage-specific genome duplications. Comparisons between *Amborella* and other angiosperms facilitated reconstruction of the ancestral angiosperm gene content and gene order in the MRCA of core eudicots. We identify new gene families, gene duplications, and floral protein-protein interactions that first appeared in the ancestral angiosperm. Transposable elements in *Amborella* are ancient and highly divergent, with no recent transposon radiations. Population genomic analysis across *Amborella*'s native range in New Caledonia reveals a recent genetic bottleneck and geographic structure with conservation implications.

The origin of angiosperms (flowering plants) prompted one of Earth's greatest terrestrial radiations, famously characterized by Charles Darwin as "an abominable mystery" (1). The oldest angiosperm fossils date from 130 to 136 million years ago (Ma), but the crown age for the angiosperms has been estimated to be at least 160 Ma (2–7). The origin of the flowering plants was followed by a rapid rise to ecological dominance before the end of the Cretaceous. Angiosperms have since diversified to at least 350,000 species, occupying nearly all terrestrial and many aquatic environments. Angiosperms provide the vast majority of human food and contribute massively to global photosynthesis and carbon sequestration. Understanding angiosperm evolution and diversification is therefore essential to elucidating key processes that underlie the assembly of biotic associations and entire ecosystems.

Paleobotany, phylogenetics, and developmental biology have dramatically reshaped views of the origin and early diversification of angiosperms (8). Most phylogenetic analyses examining chloroplast (9–12), large multigene nuclear (6, 13, 14), and chloroplast, mitochondrial, and nuclear genes combined (15) strongly support *Amborella trichopoda*, an understory shrub endemic to New Caledonia, as the single sister species to all other extant angiosperms (Fig. 1) (16). Sister lineages such as *Amborella*, when compared with other key lineages, can provide unique insights into ancestral characteristics, including genome structure and gene content. Specifically, comparisons of the *Amborella* genome reported here to other sequenced angiosperm genomes distinguish the genomic features of the most recent common ancestor (MRCA) of all extant

flowering plants from those acquired later within individual angiosperm lineages.

Genome Assembly and Annotation

The genome of *Amborella* was sequenced and assembled using a whole-genome shotgun approach that combined more than 23 Gb of single- and paired-end sequence data (~30×) obtained from multiple sequencing platforms (table S1) (17, 18). Our assembly comprises 5745 scaffolds totaling 706 Mb, 81% of an earlier genome size estimate of 870 Mb (19) and 94% of our sequence-based estimate of 748 Mb (17, 18), with a mean scaffold length of 123 kb, an N50 length of 4.9 Mb, and a maximum scaffold length of 16 Mb (table S2). Ninety percent of the assembled genome is contained in 155 scaffolds larger than 1.1 Mb.

We evaluated the quality of the assembly using an integrated strategy of comparison with available finished bacterial artificial chromosome (BAC) contig sequences (20), a BAC-based physical map (20), fluorescence in situ hybridization (FISH), and whole-genome (optical) mapping (18). Accurate and nearly complete coverage of the regions previously characterized through BAC sequencing (20) and congruence (99%) with the available physical map verify that the local contig assemblies are of high quality. FISH-based mapping of scaffold ends to chromosomes has thus far confirmed 306 Mb (44%) of the genome assembly (18).

Annotation of protein-coding genes and repetitive elements was performed with DAWGPAWS (17, 21). Despite the different histories of ancient whole-genome duplication (WGD; paleopolyploidy), the number of predicted protein-coding genes in the *Amborella* genome is similar to the number given in the most recent *Arabidopsis thaliana* reference genome annotation (TAIR10, <http://www.arabidopsis.org>). Evidence Modeler (22) was used to integrate gene annotations, producing 26,846 automated high-confidence gene predictions, 20,301 (76%) of which are supported by transcript evidence. Additionally, 17,089 gene models

contain one or more introns, with 86.9% of the splice sites supported by transcript evidence. Refined gene models were further curated through manual comparisons with *Amborella* complementary DNA transcript assemblies, gene family analyses, and homologous full-length genes from other species (17). Many of the resulting gene models included very long introns relative to other annotated genomes [for example, mean intron length is 1528 bp in *Amborella*, compared to 165, 966, and 1017 bp in *Arabidopsis thaliana*, grape (*Vitis vinifera*), and Norway spruce (*Picea abies*), respectively] (17). Annotated high-confidence protein-coding gene models occupied 152 Mb (~21.5% of the genome assembly), including 25.4 Mb of exon sequence. A conservative estimate of 17,095 alternatively spliced protein isoforms was predicted for 6407 intron-containing genes, and multiple splice site variants were inferred for 37.5% of the genes with two or more exons (17).

Gene body methylation is generally conserved in monocots and eudicots (23) and has been hypothesized to play an important regulatory role in eukaryotic genomes, distinct from the silencing of transposons (24). Whereas gene body methylation is not seen in mosses or lycophytes (25), bisulfite sequence mapping indicates that gene body methylation is prevalent in *Amborella* (fig. S5), suggesting that it is an ancestral feature found in the MRCA of flowering plants.

Angiosperm-Wide Genome Duplication

Intragenomic syntenic analysis of *Amborella* provides clear structural evidence of an ancient WGD event. An *Amborella* versus *Amborella* structural comparison shows numerous, duplicate colinear genes (syntenic homeologs) (Fig. 2A and fig. S9). Forty-seven intra-*Amborella* syntenic blocks were identified containing 466 *Amborella* gene pairs inferred to be descendants of this WGD event (Fig. 2A and table S10). Syntenic blocks contain an average of 10 homeologous gene pairs, and the longest block contains 23 gene pairs. Collectively, these 47 blocks include 6565 gene models (out of 26,846), indicating that about one-quarter of the annotated *Amborella* gene space maps to assembly scaffolds exhibiting synteny-based signal for an ancient WGD event.

Previous examinations of plant genomes have shown that polyploidy has been a prominent feature in the evolutionary history of angiosperms and that WGD events have had major impacts on genome structure and gene family evolution (7, 26–30). Although most paleopolyploid events detected to date are associated with specific angiosperm families or smaller clades, an older paleohexaploidization (genome triplication), referred to as *gamma*, has been confirmed in the common ancestor of most eudicots (26–28, 30). If the *Amborella* WGD revealed in this study was an internal, lineage-specific event, a 2:3 syntenic depth ratio would be expected between *Amborella* and *Vitis*. Instead, structural analysis shows a clear

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1:3 relationship of *Amborella* and *Vitis* syntenic blocks that map to the *gamma* paleopolyploidy (Fig. 2B and figs. S6 to S8), indicating that the WGD detected in *Amborella* is not lineage-specific and likely occurred in an ancient common ancestor of the two species, thereby confirming that the divergence of *Amborella* predates *gamma* (20, 26, 27).

Phylogenomic analyses of 11,519 gene families confirm that dispersed, duplicated genes specific to *Amborella* are uncommon (282 nontandem gene pairs), especially when compared to older gene family expansions shared across angiosperm or seed plant lineages (473 orthogroups with at least 50% bootstrap support) (17). The age distribution of the pre-angiosperm gene duplications is bimodal (fig. S17), with the two peaks corresponding to the same ancestral angiosperm (*epsilon*) and ancestral seed plant (*zeta*) genome duplications inferred in previous analyses based on transcriptome data (7). *Zeta* has escaped syntenic detection in this and other studies of angiosperm synteny, presumably because of extreme gene loss and rearrangements that have accumulated since this hypothesized ancient event more than 300 Ma.

To confirm further that the syntenic, duplicated blocks correspond to the same angiosperm-wide duplications discovered through phylogenomics, we manually curated six large duplicated blocks (Fig. 2B and fig. S10). Phylogenetic analysis of 155 syntenic gene pairs from these large blocks supports the placement of the *epsilon* genome duplication on the branch leading to the MRCA of extant angiosperms (77 of 155 gene trees resolved *epsilon* with bootstrap values of 80% or greater; see table S11).

In summary, *Amborella* genome structure demonstrates no evidence of WGD since this lineage diverged from the rest of the angiosperms at least 160 Ma. However, analyses indicate that paralogous gene copies associated with the *epsilon* WGD resulted from duplication shortly before the diversification of all living angiosperms (7). This event represents the most ancient WGD known in plants for which structural evidence persists. The *Amborella* genome therefore provides a unique evolutionary reference for elucidating genome content and structure in the MRCA of extant angiosperms and for resolving the timing of WGDs and single-gene losses and gains that

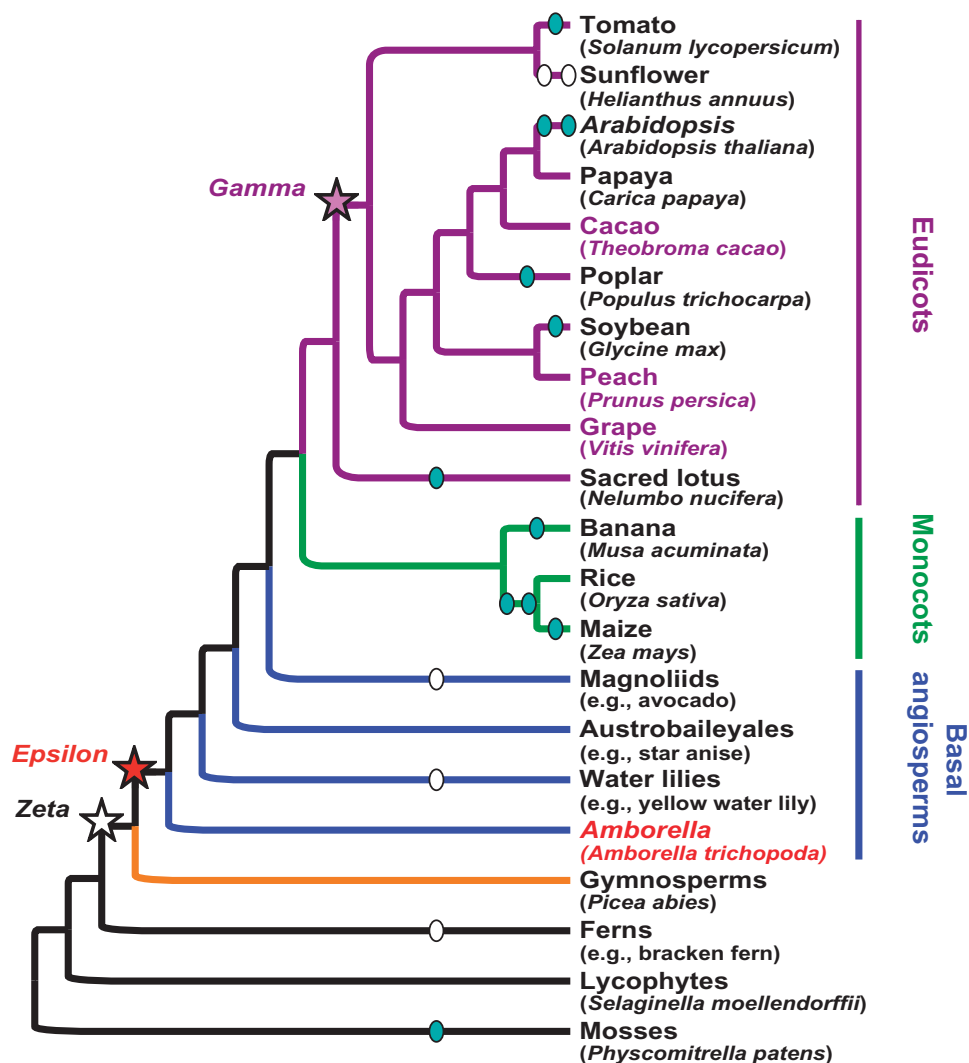
have contributed to the diversification of the angiosperms (8).

Ancestral Gene Order in Core Eudicots

We combined scaffold-level information from *Amborella* with chromosome-level data from the eudicot rosid lineages of grape (*V. vinifera*), peach (*Prunus persica*), and cacao (*Theobroma cacao*) to reconstruct the hypothetical structure of seven inferred pre-hexaploidization chromosomes in the ancestor of the core eudicots. These three species were chosen because they have retained structurally similar genomes and clear patterns of paralogy among syntenic gene copies (fig. S11), enabling us to assign most genes to one of seven groups of three homeologous chromosomes or segments (26, 27, 30, 31). A comprehensive analysis of *Amborella* and the three subgenomes from the representative rosids (combining a number of computational techniques) (29, 31, 32) enabled a completely automated reconstruction of ancestral gene order beyond the level of “contiguous ancestral regions” [compare (33)]. Figure 2C shows the orthologous gene alignments between one of the ancestral chromosomes, an *Amborella* genome

Fig. 1. *Amborella* is sister to all other extant angiosperms. An overview of land

extant angiosperms. An overview of land plant phylogeny is shown, including the relationships among major lineages of angiosperms. Representatives with sequenced genomes are shown for most lineages (Scientific names in parentheses); however, basal angiosperms (all of which lack genome sequences except for *Amborella*) and nonflowering plant lineages are indicated by their larger group names. Hypothesized polyploidy events in land plant evolution are overlaid on the phylogeny with symbols. The red star indicates the common ancestor of angiosperms and the evolutionary timing of the *epsilon* WGD (7). The evolutionary timing of *zeta* (7) and *gamma* (26, 27, 82) polyploidy events are shown with empty and purple stars, respectively. The peach, cacao, and grape genomes (purple text) were used with the *Amborella* genome to reconstruct the gene order in the pre-*gamma* core eudicot (Fig. 2C). Additional polyploidy events are indicated with ellipses. Events supported by genome-scale synteny analyses are filled, whereas those supported only with frequency distributions of paralogous gene pairs (Ks) or phylogenomic analyses are empty (34–37, 83, 84).



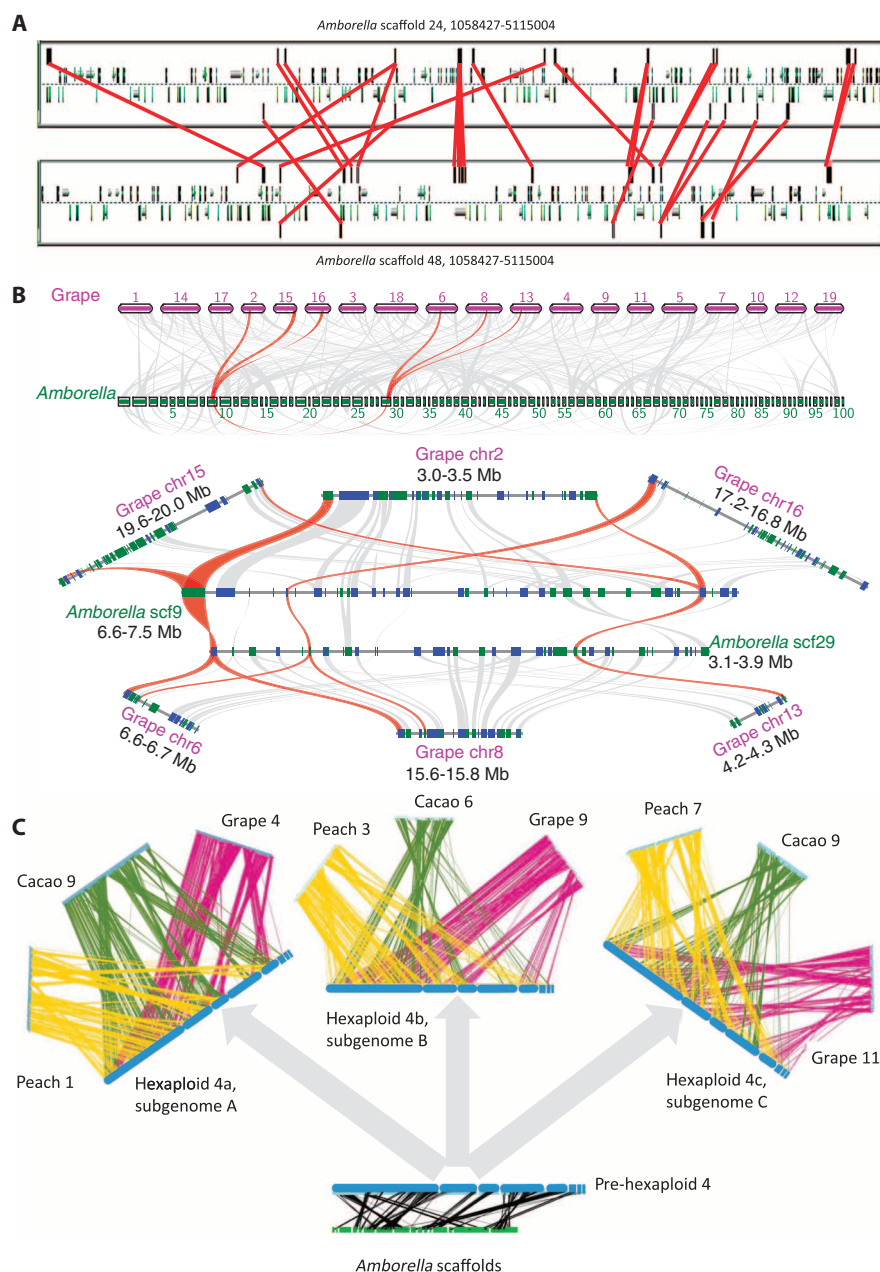


Fig. 2. Synteny analysis of *Amborella*. (A) High-resolution analysis of *Amborella*-*Amborella* intra-genomic syntenic regions putatively derived from the ancestral angiosperm (*epsilon*) WGD. Note the series of colinear genes between the two regions. Intra-genomic syntenic regions from *Amborella* are shown when scaffolds are compared and appear as a series of colinear genes between the two regions. (B) Macrosynteny and microsynteny between genomic regions in *Amborella* and grape. Top: Macrosynteny patterns between grape and *Amborella* and within *Amborella* scaffolds (only scaffolds 1 to 100 are shown). Each *Amborella* region aligns with up to three regions in grape that resulted from the *gamma* hexaploidization event in early core eudicots (27). Syntenic regions within the *Amborella* genome were derived from the *epsilon* WGD before the origin of all extant angiosperms (7). An exemplar set of blocks, showing two homeologous *Amborella* regions derived from this early WGD, aligns to three distinct grape regions (derived from *gamma*), with eight parallel regions in total. Bottom: Microsynteny is shown among the eight regions (noted above). Blocks represent genes with orientation on the same strand (blue) or reverse strand (green); shades represent matching gene pairs. (C) Gene order alignments between one of the seven hypothesized ancestral eudicot chromosomes (blue bar), the three post-hexaploidization copies of this chromosome for peach, cacao, and grape chromosomes descending from it (top of figure), and a subset of the *Amborella* scaffolds (green, bottom of figure). Similar configurations were obtained for the other six ancestral chromosomes.

scaffold, and triplicated blocks of genes in the rosid genomes. This analysis, which uses *Amborella* as an outgroup to the three eudicot genomes, would not have been possible without *Amborella* or another (as yet undiscovered) non-eudicot genome that retains a large amount of syntenic signal. The prevalence of WGDs in monocots and other basal angiosperms (34–37) limits the possibility of identifying such genomes. Similar patterns for all seven ancestral core eudicot chromosomes (17) illustrate the utility of the *Amborella* genome for reconstructing ancestral genomes within the angiosperms, thus clarifying the divergence of subgenomes after WGD events. In the case of the reconstructed core eudicot ancestor, tracking the syntenically retained descendant blocks in the three rosids reveals a consistent pattern of subgenome dominance (fig. S15). This pattern, which governs the fractionation likelihood of gene triplets generated by the *gamma* event, is not evident from the direct comparison of the extant genomes alone and highlights the value of ancestral genome reconstructions enabled by *Amborella*.

Ancestral Angiosperm Gene Content

To assess the origin and history of angiosperm genes using *Amborella* genes as an anchor, we clustered protein-coding genes from 22 sequenced land plant genomes selected for their phylogenetic representation into 53,136 orthogroup clusters (narrowly defined gene lineages; table S12), with annotations provided by the associated pfam domain and full Gene Ontology (GO) terms for genes contained in these clusters (table S16). We further merged the orthogroups into 6054 super-orthogroup clusters representing more inclusively circumscribed gene families (17). The broader circumscription of super-orthogroups allows for the clustering of more divergent homologs, thus increasing the likelihood that they represent truly distinct gene families. Phylogenetic analyses of super-orthogroups can help to root orthogroup phylogenies and resolve the relationships among related orthogroups.

We estimated the ancestral gene content at key nodes in land plant phylogeny and modeled the changes of orthogroups occurring along each branch (Fig. 3 and tables S13 to S15). The largest changes in gene family content appear to have occurred evolutionarily recently along terminal branches, or are shared among closely related taxa, such as within the tomato (*Solanaceae*) or crucifer (*Brassicaceae*) families. Large numbers of orthogroup gains were also inferred along the deeper branches leading to all angiosperms (3285 new orthogroups using parsimony reconstruction) and to grasses (4281 new orthogroups) (Fig. 3 and tables S13 to S15). However, because this analysis does not include genome sequences from ferns and gymnosperms, it cannot distinguish between orthogroups originating with euphyllophytes (ferns plus seed plants), seed plants, or angiosperms; consequently, all of these orthogroups are reconstructed along the stem branch leading to angiosperms. We sorted the inferred gene set of the recently published Norway spruce genome (38), plus gymnosperm and basal

angiosperm transcript assemblies, into this gene classification, and manually reevaluated the origin of orthogroups around the MRCAs of seed plants and angiosperms, thereby resolving or refining the origin of 5210 orthogroups, 1179 (23%) of which are specific to angiosperms or have diverged sufficiently such that none of the gymnosperm homologs were detected, with 4031 (77%) present in the MRCA of seed plants (table S13).

The large number of orthogroups first appearing in angiosperms suggests that a diverse collection of novel gene functions was likely associated with the origin of flowering plants. Analyses of GO annotations for genes in angiosperm-derived orthogroups revealed the origin of orthogroups with functions associated with key innovations defining the flowering plant clade (table S16) (17). GO annotations related to reproduction (flower development, reproductive developmental process, pollination, and similar terms), including MADS-box gene lineages (see below), were overrepresented in this set of orthogroups. Genes with roles in *Arabidopsis* floral development (table S17) are included in 201 orthogroups, 18 of which were evolutionarily derived in the MRCA of angiosperms. Significant enrichments were also observed for several classes of regulatory genes (transcription, regulation of gene expression and of cellular, biochemical, and metabolic processes) as well as genes involved in various developmental processes. These include genes involved in carpel development (*CRABS CLAW*), endosperm development (*AGL62*), stem cell maintenance in meristems (*WUSCHEL*), and flowering time (*FRIGIDA*), suggesting that they might be key components underlying the origin of the flower.

Once a functional flower evolved, genetic innovations related to reproductive biology con-

tinued. Indeed, many gene lineages with genes inferred to have specific stamen (39), carpel (39), and ovule (40) functions apparently arose after the origin of angiosperms, within evolutionarily derived angiosperm lineages (table S18).

Whereas the origin of the flower may be partly explained by novel gene lineages that first appeared with the origin of the angiosperms, other floral genes, including putative B-class (that is, petal- and stamen-specific) gene targets (41), predate the origin of angiosperms. More than 70% of the gene lineages with known roles in flowering, including genes involved in floral timing and initiation (*CO*, *SOC1*, *VIN3*, *VEL1*), meristem identity (*ULT1*, *TFL2*), and floral structure (*AFO*, *AP2*, *ETT*, *HUA2*, *HEN4*, *KAN*, *RPL*, *JAG*), were present in the MRCA of all extant seed plants (table S16) (17). Orthogroups for other major components of the floral regulatory pathway are older still, with core components of the pathway present in the ancestral vascular plant (for example, *LFY*, phytochromes, *CLV*, *SKP1*, *GAI*, *SEU*, *HEN1*, and *FVE*).

Together, these observations suggest that orthologs of most floral genes existed long before their specific roles were established in flowering, and that they were later co-opted to serve floral functions. After the origin of angiosperms, new genes originated or were recruited to refine or more narrowly parse functions associated with flower development. This pattern is consistent with the observation that the floral organ transcriptional program is canalized (entrained) in eudicots relative to the less organ-constrained transcriptomes of earlier-diverging, less species-diverse angiosperm lineages (42).

Many of the novel gene lineages that first arose in angiosperms play no specific role in reproductive processes. Orthogroups containing genes with specific functions in vessel formation

(*VND7* and *NAC083*) also first appeared at this time, even though *Amborella* does not produce vessels, but only tracheids (see below). Perhaps surprisingly, the most highly enriched GO terms in orthogroups derived in angiosperms were associated with homeostatic processes (GO:0042592; 18.9-fold enrichment). Relevant to the importance of plant-herbivore coevolution in the diversification of angiosperms and insects (43, 44), the next most highly enriched GO classification was for genes involved in response to external stimuli (GO:0009605; 10.9-fold enrichment), including those with expression elicited by herbivory.

Enrichment patterns for functional categories were similar in the ancestral seed plant and ancestral angiosperm (table S16), including novel lineages of genes involved in reproductive, regulatory, and developmental processes. GO classifications associated with pollen-pistil interaction and epigenetic modification were enriched in orthogroups arising on the branch leading to seed plants, but not in the lineage leading to the ancestral angiosperm (table S16), the former perhaps indicating that some angiosperm-specific reproductive features predated angiospermous (enclosed ovule) reproduction.

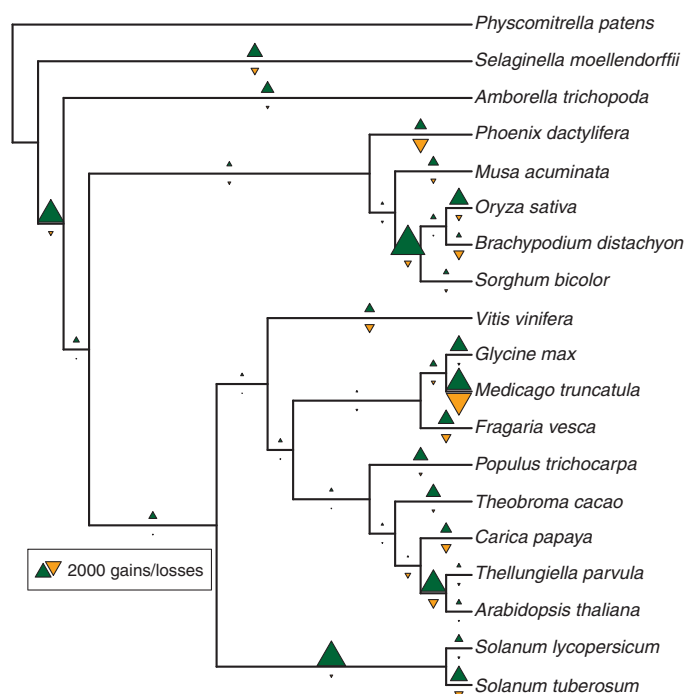
Gene Family Expansions in Angiosperms

Expansions of many gene families are evident in *Amborella*, and phylogenetic analyses indicate that such expansions occurred in the ancestral angiosperm, accompanying innovations associated with angiosperm origin. Using *Amborella* as a reference, we examined patterns of gene family diversification in angiosperm evolution, often in association with phenotypic divergence among angiosperm lineages.

MADS-Box Genes

MADS-box transcription factors are among the most important regulators of flower development. The *Amborella* genome encodes 36 MADS-box genes (table S19) (17), fewer than in other angiosperms (for example, *Arabidopsis* and rice), but consistent with the lack of a lineage-specific WGD. These genes belong to 21 clades, each of which includes genes from at least one other major lineage of angiosperms, implying that a minimum set of 21 MADS-box genes existed in the MRCA of extant angiosperms (figs. S19 and S20). The *Amborella* genome reveals that floral organ identity genes from eight major lineages (that is, *AP1/SQUA*, *AP3/DEF*, *PI/GLO*, *AG*, *STK*, *AGL2/SEP1*, *AGL9/SEP3*, and *AGL6*; Fig. 4A) existed in the MRCA of extant angiosperms and were likely derived from three ancestral lineages in the MRCA of extant seed plants. These data support the hypothesis that duplication and diversification of floral MADS-box genes likely occurred before the origin of extant angiosperms, despite being tightly associated with the origin of the flower. Furthermore, the previously presumed monocot-specific *OsMADS32* and eudicot-specific *TM8* gene lineages (fig. S20) (45–47) have orthologs in *Amborella*, suggesting that they were likely present in the earliest angiosperms and were subsequently lost in eudicots or monocots, respectively.

Fig. 3. Ancestral reconstruction of gene family content in land plants. Orthogroup gains and losses are inferred from the global gene family classification of proteins from sequenced plant genomes using a Wagner parsimony framework (17). Triangles are proportional to the number of orthogroup gains (green) and losses (orange). Actual values for the gains and losses in this analysis are provided in table S14; an analogous likelihood-based analysis is provided in table S15.



MADS-box transcription factors in floral development form dimers or higher-level complexes that bind to their targets with more complex patterns than those in gymnosperms (48). We conducted a comprehensive series of yeast two-hybrid assays among the *Amborella* floral MADS-box transcription factors. The protein-protein interaction (PPI) patterns in *Amborella* (fig. S21) are generally consistent with those in other angiosperms, and show clear differences from those in gymnosperms. For example, the B-function *AP3/DEF* and *PI/GLO* genes represent duplicate lineages in early angiosperms, arising after the divergence from the gymnosperms. The *Amborella* AP3 and PI homologs form heterodimers, as in other angiosperms, whereas the single AP3/PI homologs in gymnosperms form only homodimers, with heterodimers only occurring between recent duplicates of the AP3/PI homologs (Fig. 4B). B function is essential for the development of petals and other petal-like organs, which represent one of the most prominent novel floral features and exhibit extraordinary diversity in form; therefore, evolutionary shifts in PPI patterns after gene duplications, along with changes in gene sequence and expression patterns, likely have been crucial for functional innovations in the regulatory network for reproductive organ development and the origin of the flower (49), as well as for functional diversification of the many floral forms among lineages of angiosperms (50).

Glycogen Synthase Kinase 3 (GSK3) genes

GSK3 genes encode signal transduction proteins with roles in a variety of biological processes in eukaryotes. In contrast to their low copy numbers

in animals, *GSK3* genes are numerous in land plants and have diverse functions, including floral development in angiosperms (51). Five *GSK3* loci that were present in the ancestral angiosperm have subsequently diversified among major angiosperm lineages, but a sixth ancestral locus has been detected only in *Amborella* (fig. S22). Thus, among flowering plants, *Amborella* alone may contain all the *GSK3* gene lineages that arose before the origin of extant angiosperms, underscoring the importance of *Amborella* for reconstructing the ancestral angiosperm genome (52).

Seed Storage Globulins

Seed storage proteins, including globulins, are critical for embryo and early seedling development in seed plants. These proteins are embedded in the very diverse cupin superfamily, which is distributed across the tree of life (53). The 11S legumin-type globulins are widespread across the seed plant phylogeny [for example, (54–56)]. Three distinct 11S legumin-type globulins have been identified in proteomic analyses of the globulin fraction in *Amborella* seeds (table S21) (17). Comparisons of the *Amborella* globulin-coding gene sequences to other seed plants revealed that key cysteine residues contributing to disulfide bonding between subunits and the absence of Intron IV, found in gymnosperms, are conserved characteristics of angiosperm legumins (fig. S25). In contrast, a conserved 52-residue region present in soybean, and thought to be important for mature hexamer formation (57), was apparently derived after the divergence of *Amborella* from other angiosperms (fig. S26). Globally, both structural (fig. S26) and phylogenetic (fig. S27 and

table S22) analyses support the view that *Amborella* 11S globulins can both be reminiscent of those in monocots and eudicots and exhibit specific features of corresponding seed storage proteins in basal angiosperms and gymnosperms.

Terpene Synthase Genes

Terpenoids constitute the largest class of plant secondary metabolites and play important roles in plant ecological interactions (58). Biosynthesis of plant terpenoids is driven by terpene synthases (TPS). The *Amborella* TPS family contains more than 30 members, comparable in size to those of other angiosperms. However, the sesquiterpene synthase subfamily a (TPS-a), which is present in dicots, monocots, and Magnoliaceae but absent in gymnosperms and non-seed plants (59), is also absent in *Amborella* (fig. S28) (17). This indicates that the occurrence and diversification of this subfamily likely happened after the divergence of *Amborella* from other angiosperms, although its presence or absence in other basal angiosperms still needs to be established. Sesquiterpene synthases are involved in the production of C15 terpenoids, which are involved in diverse biological processes including the production of floral scents used to attract pollinators. *Amborella* lacks any detectable floral volatiles (60), and the expansion of the TPS-a subfamily may therefore have played an important role in the subsequent radiation of flowering plants.

Cell Wall and Lignin Genes

Secondary cell walls of woody plants contain lignin (61), facilitating water transport and mechanical support in xylem (62). Most gymnosperms (cycads,

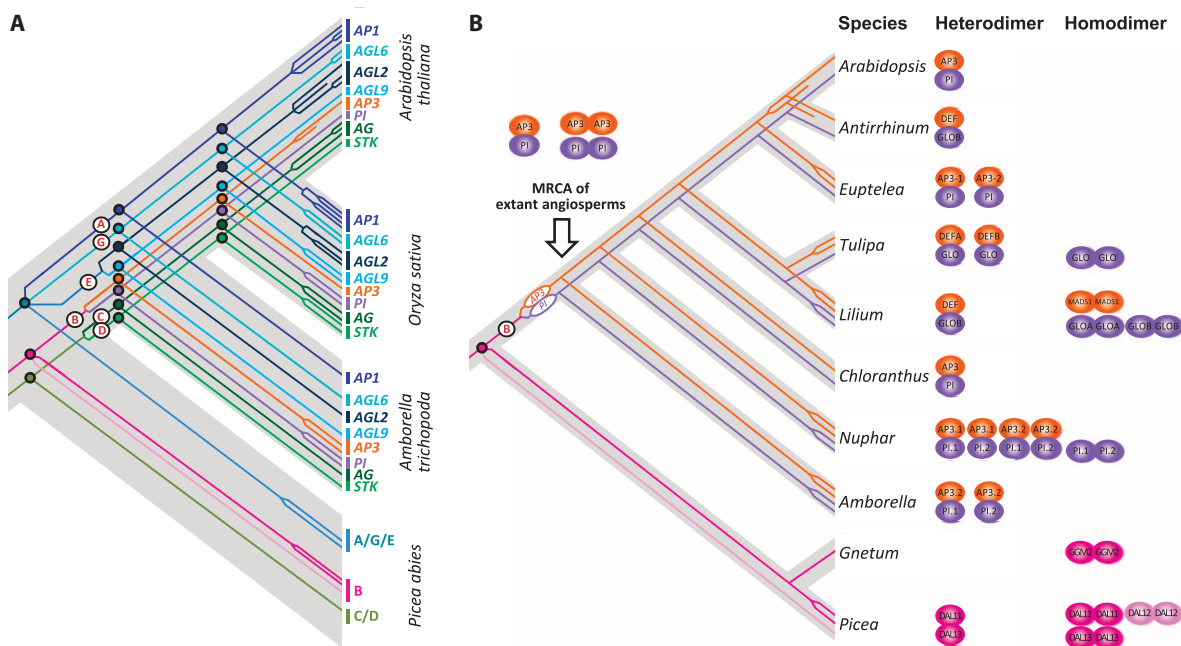


Fig. 4. *Amborella* as the reference for understanding the molecular developmental genetics of flower evolution. (A) A schematic diagram showing the evolutionary history of floral MADS-box genes. Note that all of the eight major gene lineages existed in the MRCA of extant angiosperms. (B) Evolutionary changes in the ability of B-class MADS-box proteins to form homodimers and

heterodimers. In gymnosperms, the proteins of B-class genes can only form homodimers or semi-homodimers (that is, heterodimers formed by products of recently duplicated genes), whereas in the MRCA of extant angiosperms, they gained the ability to form heterodimers between members of different lineages. The interrupted lines represent previously described gene loss events (85, 86).

Ginkgo, and conifers) have a predominant guaiacyl (G) subunit type of lignin, whereas the gnetophytes (<100 species) and the woody angiosperms have a lignin characterized by a copolymer of syringyl S and G subunits (S/G lignin). S/G lignin has also been found in the lycophyte *Selaginella moellendorffii*, suggesting that S/G lignin may have evolved more than once in plant evolution (63). S/G lignin is associated with cells involved in mechanical support, whereas G-type lignin has been associated with water transport (64). *Amborella* produces an S/G type of lignin, but the relative proportion of S subunits is much lower (13%) than values typical of woody angiosperms (50 to 70%) (tables S24 to S26). The low S/G ratio of *Amborella* might represent an ancestral condition that was transitional between gymnosperms and other angiosperms. However, the underlying genes of lignin precursor biosyn-

thesis in *Amborella* are typical of woody angiosperms (table S27 and fig. S29, A to H). Although *Amborella* lacks vessels, in contrast to nearly all other angiosperms (65), the wood cell walls of *Amborella* are xylan-rich (table S24), typical of angiosperms (66). *Amborella* contains all of the carbohydrate-active enzyme families found in angiosperms (table S28) (67), but lacks many of the more derived clades of genes seen in other angiosperms. Indeed, much of the diversity of cell wall genes in angiosperms (for example, glycosyltransferase family 37; fig. S25) appears to result from gene duplication after the divergence of *Amborella* from other angiosperms.

Transposable Element Content in *Amborella*

As in the Norway spruce genome (38), the average age of identifiable transposable elements

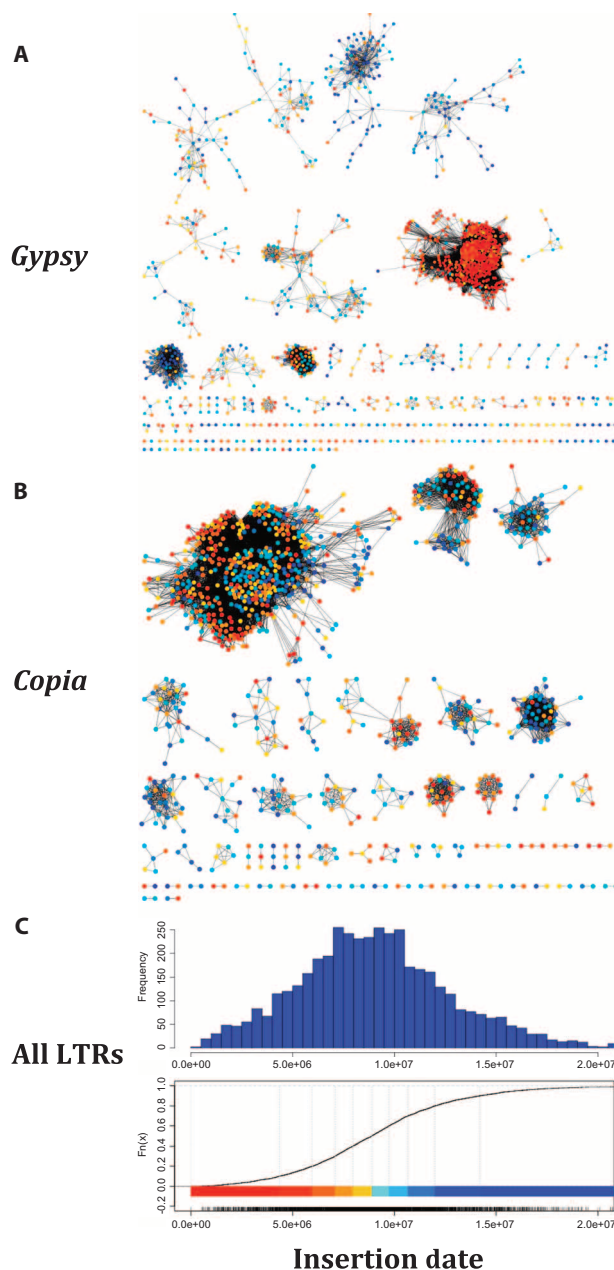
(TEs) in *Amborella* is considerably older than that of other angiosperm genomes [for example, (68–70)]. Likewise, ancient, full-length long terminal repeat (LTR) retrotransposons were identifiable in *Amborella* more than an estimated 40 million years after insertion (17). Wicker *et al.* (71) established the convention of separating LTR retrotransposons exhibiting more than 80% divergence in their terminal repeats into distinct families, but nearly 10% of individual *Amborella* LTR elements show a greater degree of divergence between their terminal repeats. Therefore, we used a clustering approach to circumscribe TE families. Median estimated insertion times for LTR subfamilies with two or more detectable TEs ranged from 4.0 to 17.6 Ma. A large class of *Gypsy* LTR retrotransposons with 502 annotated TEs experienced the most recent burst of activity 0.5 Ma (Fig. 5) (17). Endogenous pararetroviruses (EPRVs) were a relatively large component of the repeat landscape, comprising 2.4% of the assembled *Amborella* genome. Similar to *Sorghum bicolor*, which has a comparable genome size, TEs and EPRVs account for 57.2% of the nonambiguous nucleotides in the *Amborella* genome (668 Mb, table S30), but TE insertion times estimated for the *Amborella* genome are much older than inferred for *Sorghum* (64). Only four of the common superclasses of DNA TEs were observed (table S30); CACTA and TC1/Mariner-type elements were not detected. Most DNA TEs were highly degraded, with highly divergent sequences and missing terminal inverted repeats, again suggesting the persistence of identifiable elements over millions of years. The lack of recent transposon activity in the *Amborella* genome may be due to very effective silencing or the loss of active transposases.

Evolution of Small RNAs

More than 56,000 discrete loci generating apparent regulatory small RNAs 20 to 24 nucleotides (nt) in size were identified by analysis of small RNA-seq data (17). Most small RNA loci had features consistent with those of heterochromatic small interfering RNAs (siRNAs) (24), indicating that heterochromatic siRNAs were present in the MRCA of all angiosperms. We also identified 124 *MIRNA* loci corresponding to 90 distinct families; 27 of these microRNA (miRNA) families, including 5 newly discovered ones, were likely present in the ancestral angiosperm. Most of these families (19 of them) are broadly conserved in other angiosperms, whereas 8 have evidence suggestive of later losses during angiosperm diversification. Inferred targets of the ancestral miRNA families were generally homologous to known miRNA-target relationships in other angiosperms, demonstrating that these relationships have been conserved since the earliest angiosperms despite the one-to-several rounds of polyploidy that separate *Amborella* from most other flowering plants. The other 63 miRNA families appear to be lineage-specific, and we could verify targets for just 14 of them. Surprisingly, most (78%) of

Fig. 5. Classification and insertion dates of LTR transposons in the *Amborella* genome. *Gypsy* (A) and *Copia* (B) LTR transposons are clustered into putative families, and individual elements are colored by their estimated insertion dates.

Cool colors (for example, blue) represent older insertions, whereas warm colors (for example, red) represent more recent insertion dates. (C) Although some LTR transposon families have been active over the last 5 million years (for example, the large *Gypsy* cluster), the estimated insertion dates for the majority of elements are more than 10 Ma. See (17) for median insertion dates for each cluster (table S29s).



these lineage-specific miRNAs were 23 to 24 nt in size, rather than the 20- to 22-nt size typical of plant miRNAs. In contrast, none of the conserved miRNAs were 23 to 24 nt in size. The frequency of 23- to 24-nt miRNAs in *Amborella* is higher ($>2\times$) than for any other land plant reported. Similar to the results for *Medicago* (72) and members of Solanaceae (73, 74), several phased siRNA loci were nucleotide binding site–leucine-rich repeat (NB-LRR) disease resistance genes targeted by miRNAs in the miR482/2118 superfamily. Therefore, phased siRNA production from NB-LRR genes was likely present in the MRCA of angiosperms.

Population Genomics and Conservation Implications

Amborella is restricted to wet tropical forests on isolated slopes of New Caledonia. The genomes of 12 individuals of *Amborella*, sampled from nearly all known populations, were resequenced to assess the levels and patterns of genetic variation within this endemic species. These 12 individuals harbor levels of genetic diversity ($\theta_w = 0.0017$, $\pi = 0.0021$) similar to those reported for species of *Populus*, which are also outcrossing perennials (table S45). The average Tajima's *D* across the genome (75) is positive ($D = 0.8137$), perhaps indicating balancing selection, although demographic processes such as population subdivision,

a recent bottleneck, or migration can also produce a positive value. However, the genome exhibits significant among-locus and among-scaffold variance in allelic variation (fig. S40). Some regions, such as scaffold 1, are highly polymorphic and heterogeneous across their length, whereas other regions are nearly invariant with negative Tajima's *D* (for example, scaffold 31; fig. S40), consistent with multiple alternative explanations, such as recent selective sweeps and/or a mixed mating system.

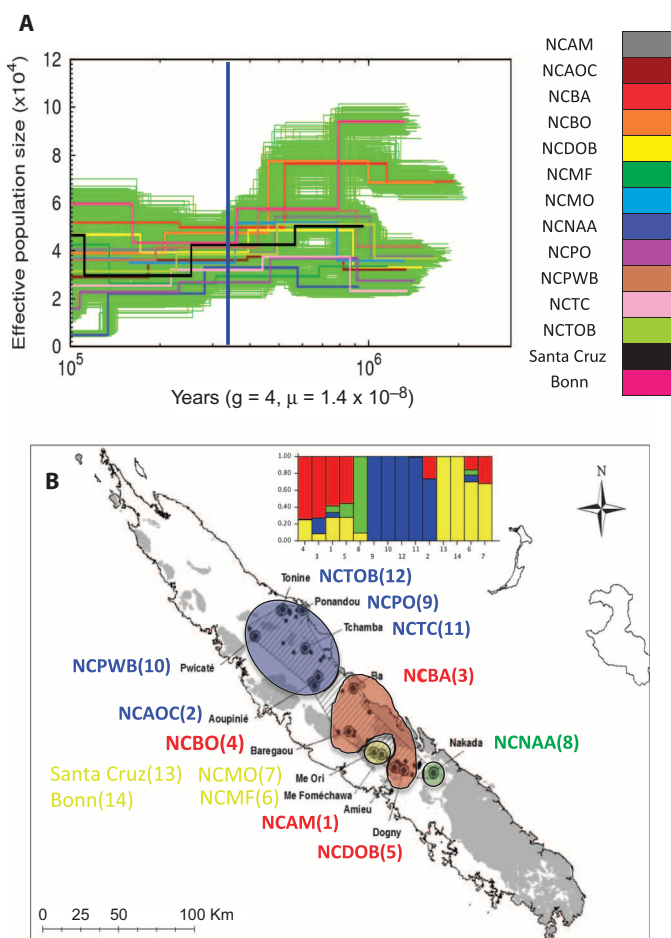
The overall positive value of Tajima's *D* is consistent with a decrease in population size through time, as also demonstrated by an analysis of population genomic history using the pairwise sequentially Markovian coalescent (PSMC) (76) model, which has recently been applied to plant genomes (77). PSMC analysis of all 14 *Amborella* individuals, including the reference genome, the cultivated Bonn specimen, and the 12 locality-specific exemplars (Fig. 6A), reveals that the variation present in these modern genomes coalesces between 0.9 and 2 Ma. Confidence intervals for PSMC analyses of each individual are consistent with the hypothesis that at least two distinct *Amborella* sublineages with different levels of genetic diversity converged by 800,000 years ago, followed by admixture and a subsequent bottleneck event between 300,000 and 400,000 years ago, and by some recovery of genetic diversity thereafter. *Amborella* may therefore have undergone a

series of population bottlenecks over the past 900,000 years, including one as recent as 100,000 years ago, represented by individual NCNAA (Fig. 6A). At the time of putative sublineage admixture (vertical line), effective population size (N_e), as averaged among all sequenced accessions, approximated 37,500 individuals, whereas in the recent event in NCNAA's past (where the PSMC plot reaches the ordinate axis), N_e may have been much lower at 5000 individuals or less (Fig. 6A). The reduction in N_e associated with any of these bottlenecks could have contributed to increased genetic structure among populations and linkage disequilibrium (LD). Increased LD may contribute to the size and persistence of genomic regions affected by selective sweeps, if they have occurred. Further analyses, with greater population sampling, are needed to distinguish the relative roles of selection, inbreeding, and other processes in shaping genome variability in *Amborella*.

Genetic variation among *Amborella* populations is significantly structured into four geographic clusters of populations on New Caledonia (Fig. 6B), corresponding roughly to populations in (i) the northern part of the range (blue cluster), (ii) the central part of the range (red cluster), (iii) a small region west of cluster 2, and (iv) a single disjunct location at the southern end of the distribution. These results are consistent with an independent analysis and extensive sampling of the 12 populations using microsatellite loci (78). Population genomic analyses tell a tale of dynamic genome evolution in this narrowly distributed plant species, the sole extant member of a lineage that shared a common ancestor with all other extant angiosperms about 160 Ma. Despite its restricted distribution, *Amborella* maintains substantial genetic diversity, with substructure among four population clusters. As ongoing effects of an expanding human population (for example, mining operations, fires, urbanization, and invasive species introduction) threaten the unique flora of this biodiversity hotspot, conservation efforts in New Caledonia should focus on preserving and managing the genetic diversity of New Caledonia's endemic species, including *A. trichopoda*.

Fig. 6. Population genomic diversity in *Amborella*.

(A) Plots of PSMC results for 14 individuals: 12 from separate populations on Grande-Terre, New Caledonia, the reference genome (Santa Cruz), and an additional cultivated individual (Bonn), indicated in the color panel (right) and with bootstrap clouds (for each genome analyzed) co-plotted in green. Times more recent than 10^5 years, where PSMC can be less reliable, are excluded. A vertical bar is drawn over the plot at about 325,000 years before present to indicate the timing of species-wide decline of effective population size, interpreted as a genetic bottleneck. (B) Results of STRUCTURE analysis, showing four significant genetic clusters of 12 individuals from natural populations. Additionally, the cultivated individual from the Bonn Botanical Garden and the reference genome (Santa Cruz) are clustered with individuals from Mé Foméchawa and Mé Ori [see (17)].



Conclusions

The phylogenetic position, conservation of genome structure, and absence of a lineage-specific polyploidy event have made the *Amborella* genome a unique and valuable reference that facilitates interpretation of major genomic events in flowering plant evolution, including the polyploid origin of angiosperms and a genomic hexaploidization event in eudicots. *Amborella* has enabled the identification of an ancestral gene set for angiosperms of at least 10,088 genes, including many that resulted from the ancestral angiosperm genome duplication, thereby helping to elucidate the origin of genes critical in flowering and other processes. The ancestral angiosperm-wide genome duplication apparent in the *Amborella* genome not only serves as a genetic marker for the origin of extant angiosperms, but it may also have set in motion a

series of events as numerous genes evolved novel functions, eventually leading to modern flowering plants. As the only extant member of an ancient lineage, *Amborella* provides a unique window into the earliest events in angiosperm evolution.

Materials and Methods

Sequencing and Assembly

Plant material for the reference genome sequence was obtained from a plant in cultivation since 1975 at the University of California at Santa Cruz Botanical Garden and additional clones located at the Atlanta Botanic Garden and the University of Florida. Single end genomic 454-FLX and SE 454-FLX+, DNA sequences, 11-kb paired-end 454-FLX reads, 3-kb PE Illumina HiSeq reads, and Sanger sequenced BAC end sequence reads were filtered to remove organellar contaminants, reads of short length or poor quality, artificial duplicates, and chimeras. After filtering, the read collection was pooled and assembled with the Roche Newbler assembler V2.6 [see (18) for details].

Genome Annotation and Database Development

Protein-coding genes, transposons, and endogenous viral sequences within the assembled genome were annotated iteratively using a variety of homology-based and de novo prediction algorithms integrated within the DAWGPAWS package (21). Initial gene model and transposon annotations were curated, and refined models were used to train ab initio prediction programs. The PASA annotation pipeline (79) was used to identify and classify alternative splicing events by aligning Newbler assembled 454 and Sanger expressed sequence tags (ESTs) and Trinity RNA-Seq assemblies. Three small RNA libraries and two degradome libraries were sequenced and used for annotation of small RNA-producing loci (including miRNAs, phased siRNAs, and heterochromatic siRNAs) and their targets. All resulting gene and transposon predictions, as well as alternative splicing annotations, have been placed in appropriate databases accessible through the *Amborella* Genome Database (<http://www.amborella.org/>) and National Center for Biotechnology Information (NCBI) (BioProject PRJNA212863).

Cytogenetics

Fluorescently labeled BACs were applied to mitotic chromosome spreads from root tips following Kato *et al.* (80). A Zeiss Axio Imager.M2 fluorescence microscope with an X-Cite Series 120 Q Lamp (EXFO Life Sciences) was used for visualization, and images were captured with a 100× objective lens and a microscope-mounted AxioCam MRm digital camera (Zeiss) in conjunction with Axiovision version 4.8 software (Zeiss).

Syntenic Analyses

For uncovering within-genome WGDs, we used the SynMap tool in the online CoGe portal ([http://](http://genomeevolution.org/CoGe/)

genomeevolution.org/CoGe/), specifying a minimum number of colinear genes per window size to define putative syntenic regions. These regions were subsequently compared and confirmed using the microsynteny tool GEvo, also in CoGe. Blocks determined to represent the pan-angiosperm duplication event were further studied using phylogenomic methods to ascertain whether duplication patterns on trees concurred with a region-wide duplication model.

Scaffolds containing up to 10 orthologous and paralogous genes in common syntenic context from *Amborella* and three *gamma* subgenomes of three rosids were ordered using maximum weight matching to produce a hypothetical ancestral core eudicot genome with seven chromosomes. Each of the subgenomes mapped to virtually the whole length of the appropriate reconstructed chromosome. The reconstructed genes show a much clearer pattern of pan-rosid fractionation bias in extant genomes than is apparent without evidence derived from the *Amborella* genome scaffolds.

Global Gene Family Circumscription and Analysis

A global plant gene family classification was created using OrthoMCL (81) for the annotated protein set of *Amborella* and 21 other land plant genomes. The gene families (orthogroups) were populated with the gene models from the Norway spruce genome and a large collection of EST assemblies from basal angiosperms and other gymnosperms. We analyzed the evolutionary history of gain and loss of orthogroups and estimated the gene families present in the MRCA of living angiosperms using both parsimony and likelihood methods. Genome-wide analyses were performed, as well as more focused studies of genes with roles in flower development.

To study the history of ancient gene duplications in angiosperms and seed plants, we performed maximum likelihood phylogenetic analysis of 11,519 orthogroups that contained *Amborella* genes. Gene duplications were scored on the basis of taxa present in the daughter lineages to identify angiosperm-wide, seed plant-wide, and *Amborella*-specific gene duplications (7). Possible genome duplications were identified from statistically significant peaks in the distributions of synonymous divergences and estimated ages of gene duplication events. Six of the largest syntenic blocks in the *Amborella* genome were also used for manual curation of syntenic duplicates and phylogenetic analysis of gene families containing duplicated genes present on paralogous genomic blocks.

Targeted Gene Family Analyses

To illustrate the value of the *Amborella* genome as a reference for understanding the evolutionary history of gene families associated with angiosperm innovations or divergence among angiosperm lineages, we examined the phylogenetic history of MADS-box, GSK3, TPS, and cell wall

and lignin genes. Yeast two-hybrid analysis of MADS-box proteins in *Amborella* was used to identify heterodimeric PPIs found only in angiosperms. Proteomic and phylogenetic analysis of seed storage globulin proteins validated protein-coding gene models as well as examined protein features that separate angiosperms from earlier land plant lineages.

Population Genomics

To assess the levels and patterns of genetic variation within *A. trichopoda*, we sequenced the genomes of 12 individuals representing nearly all of the known natural populations of the species, the reference plant, and an additional accession from the Bonn Botanical Garden. Sequences were mapped to the reference genome using BWA. We used basic population genetic measures to infer levels of diversity and applied the PSMC model, originally applied to human and other mammalian genomes, to study the effective population size (N_e) of *Amborella* over time. Genetic divergence among populations was assessed using STRUCTURE.

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Authorship information

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Supplementary Materials

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Text

Figs. S1 to S42

Tables S1 to S46

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References

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Horizontal Transfer of Entire Genomes via Mitochondrial Fusion in the Angiosperm *Amborella*

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We report the complete mitochondrial genome sequence of the flowering plant *Amborella trichopoda*. This enormous, 3.9-megabase genome contains six genome equivalents of foreign mitochondrial DNA, acquired from green algae, mosses, and other angiosperms. Many of these horizontal transfers were large, including acquisition of entire mitochondrial genomes from three green algae and one moss. We propose a fusion-compatibility model to explain these findings, with *Amborella* capturing whole mitochondria from diverse eukaryotes, followed by mitochondrial fusion (limited mechanistically to green plant mitochondria) and then genome recombination. *Amborella*'s epiphyte load, propensity to produce suckers from wounds, and low rate of mitochondrial DNA loss probably all contribute to the high level of foreign DNA in its mitochondrial genome.

Many of the fundamental properties of eukaryotes arose from horizontal evolution on a grand scale—that is, the endosymbiotic origin of the mitochondrion and plastid from bacterial progenitors (1). Since their birth, however, mitochondrial and plastid genomes seem to have been little affected by horizontal gene transfer (HGT). The most notable exception involves land plants, especially flowering plants (angiosperms), in which HGT is common in the mitochondrial genome but unknown in plastids (2–10).

To gain insight into the causes and consequences of HGT in mitochondrial DNA (mtDNA), we sequenced the mitochondrial genome of *Amborella trichopoda* because polymerase chain reaction–based sampling had shown it to be rich in foreign genes (4). This large shrub is endemic to rain forests of New Caledonia and is probably sister to all other angiosperms, a divergence dating back about 200 million years (11, 12).

Overall Genome Properties

The *Amborella* mitochondrial genome assembled as five autonomous, circular-mapping chromo-

somes of lengths 3179, 244, 187, 137, and 119 kb, giving a total genome size of 3,866,039 base pairs (bp) (Fig. 1A and figs. S1 to S4) (13). The five chromosomes are distinct in sequence but similar in base composition (45 to 47% G + C), stoichiometry, and HGT properties (Fig. 1A and figs. S2 and S4). Stoichiometry was assessed by sequencing coverage and Southern blot analysis of 32 individuals from three populations (fig. S5) (13).

As described in the next three sections, *Amborella* mtDNA possesses an extensive and diverse collection of foreign sequences, corresponding to about six genome equivalents of mtDNA acquired from mosses, angiosperms, and green algae. Multigene HGT has been described in two other lineages of plant mtDNA (8, 10), but not on a scale approaching *Amborella*. The *Amborella* mitochondrial genome also contains a large amount (138 kb) of plastid DNA (ptDNA) (Fig. 1A, fig. S2, and table S1).

Multichromosomal mitochondrial genomes in plants were only recently discovered (14, 15) and mostly involve large (>1 Mb) genomes, with *Silene* genomes of 6.7 and 11.3 Mb dwarfing *Amborella* in size and chromosome number (15). These three mitochondrial genomes are the largest completely assembled organelle genomes, larger than many bacterial genomes and even some nuclear genomes. However, the processes responsible for their expansion differ in that *Silene* genomes possess no readily discernible foreign mtDNA and relatively little ptDNA (15).

HGT from Mosses

Amborella mtDNA contains four regions, of lengths 48, 40, 9, and 4 kb, acquired from moss mtDNA (Fig. 1A and fig. S2). With one exception, the 41 protein and ribosomal RNA (rRNA) genes from these four regions were placed phylogenetically, almost always strongly, as sister to the moss *Physcomitrella* (Fig. 2, A to D, and figs. S8 and S9). Gene order in the four regions (Fig. 3 and

fig. S6) is highly similar to both *Physcomitrella* and *Anomodon* (mosses that are themselves identical in gene order and content) (16) and extremely different from angiosperms. The mosslike regions in *Amborella* also harbor the same 27 introns and largely the same set of intergenic sequences as moss mtDNAs (fig. S6) (13).

The four moss regions contain one, and only one, copy of 61 of the 65 genes present in sequenced moss mtDNAs (Fig. 3 and fig. S6) (13). Taking into account six inferred deletions and duplications larger than 100 bp, the 101.8 kb of moss DNA in *Amborella* reconstructs to a hypothetical donor genome of 106.0 kb, compared with the 104.2- and 105.3-kb genomes in *Physcomitrella* and *Anomodon*, respectively. We infer, therefore, that *Amborella* captured an entire mitochondrial genome (13) from a moss with nearly identical mtDNA architecture to those of *Physcomitrella* and *Anomodon*. This foreign genome subsequently rearranged into four pieces, with a few gene-order changes and 11 gene losses, truncations, and/or partial duplications, all of which are associated with rearrangement breakpoints (Figs. 1A and 3, figs. S2 and S6, and table S2).

HGT from Green Algae

The *Amborella* mitochondrial genome contains an average of three green algal–derived copies of each protein and rRNA gene commonly found in green algal mtDNAs (Figs. 1A and 2, A to D; figs. S2, S4, S8, S10, and S11; and table S3). Many of these genes are clustered in two large tracts of lengths 83 and 61 kb. The 83-kb tract (B1 + A2 in Fig. 1A) contains two copies of a 10-gene cluster (each marked by 10 red arrows in the top comparison of Fig. 4), with all 10 “duplicates” highly divergent from each other. The 61-kb tract (B2 + A1 in Fig. 1A) lacks these 10 genes and instead contains highly divergent duplicates of two genes that are absent from the 83-kb tract. A single hypothesized recombination event between these two tracts (Figs. 1A and 4) accounts for the above duplications, with the initial, 92- and 52-kb regions each containing a nearly complete set of green algal mitochondrial genes and no extra copies (fig. S11). We conclude that the 83-kb and 61-kb tracts arose by acquisition of whole mitochondrial genomes (designated the A and B genomes) from two green algae, followed by a single recombination between them and a few gene losses (13). Additionally, the two inferred donor genomes are phylogenetically distinct: Whenever *Amborella* has three or more green algal copies of a given gene, the A-genome copy is separated by a relatively long branch from a well-supported clade containing the other green algal copies (Fig. 2, A and D, and fig. S8). Furthermore, the two regions assigned to the A genome have a lower noncoding G + C composition (39%) than the two B-genome regions (47%) (table S4).

Most of the remaining green algal mtDNA in *Amborella*, comprising tracts of lengths 49, 18, 16, and 2 kb (Fig. 1A and fig. S2), also appears,

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on the basis of synteny and genome reconstruction (Fig. 4 and fig. S11), to be derived by whole-genome transfer (from donor C). Seven of the eight remaining, mostly short tracts of green algal mtDNA (Fig. 1A) can tentatively be reconstructed as resulting from the transfer and/or retention of about one-third of a genome from a fourth green algal donor (donor D); alternatively, the D regions may result from multiple HGT events. Although the B, C, and D genomes are relatively similar in sequence (Fig. 2, A to D, and fig. S8), their many differences in gene order (Figs. 1A and 4) and intron content (e.g., *cox1* has two introns in the D genome but none in B) rule out the possibility that they result from only one or two transfers followed by large-scale duplication within *Amborella*. We therefore conclude that *Amborella* acquired its ~3.3 genome equivalents of green algal mtDNA (Fig. 1A) through at least four transfers, including three whole-genome transfers.

The multiple copies of each green algal gene present in *Amborella* almost always ally, usual-

ly strongly, with the trebouxioophyte *Coccomyxa* (Fig. 2, A to D, and fig. S8). Likewise, gene order within the A, B, and C genomes is most similar to that of *Coccomyxa* (fig. S7). The B, C, and D copies of each gene invariably form a strongly supported clade (Fig. 2, A to D, and figs. S8 and S10), with the B + C genomes sister to the A genome in gene-loss phylogeny (fig. S12). Thus, *Amborella* probably acquired its green algal mtDNA from the *Coccomyxa* subgroup of trebouxioophytes. Because members of this subgroup often live as lichen photobionts, and lichens commonly grow on *Amborella* (Fig. 5), its algal genomes may have been acquired from lichens.

HGT from Angiosperms

Amborella mtDNA contains 150 angiosperm-like copies (full or partial) of the 49 protein and rRNA genes likely present in the ancestral angiosperm mitochondrial genome (fig. S13) (17) [see (13) for how trans-spliced genes are counted (table S5)]. We designated 82 of these copies as foreign, 63 as

native, and 5 as uncertain (table S3). Angiosperm-specific phylogenetic analyses provided strong support for 26 (32%) of the foreign assignments and 16 (25%) of the natives and lower support for an additional 20 foreign and 22 native assignments (figs. S14 to S16 and tables S6 and S9). The lower support values reflect the generally poor resolution in many of the trees (fig. S14), which is a consequence of low substitution rates in most angiosperm mtDNAs (18).

Four other lines of evidence were used to distinguish foreign from native angiosperm genes and intergenic DNA. First, the extent of cytidine to uridine (C-to-U) RNA editing, which is much higher in *Amborella* than in all examined eudicots and monocots (table S7) (13), provided evidence for native versus foreign origin for many of the 150 angiosperm genes in *Amborella* mtDNA (13). Second, six genes were exceptionally divergent relative to all other genes analyzed phylogenetically (fig. S15), suggesting that they came from angiosperms with much higher mtDNA substitution

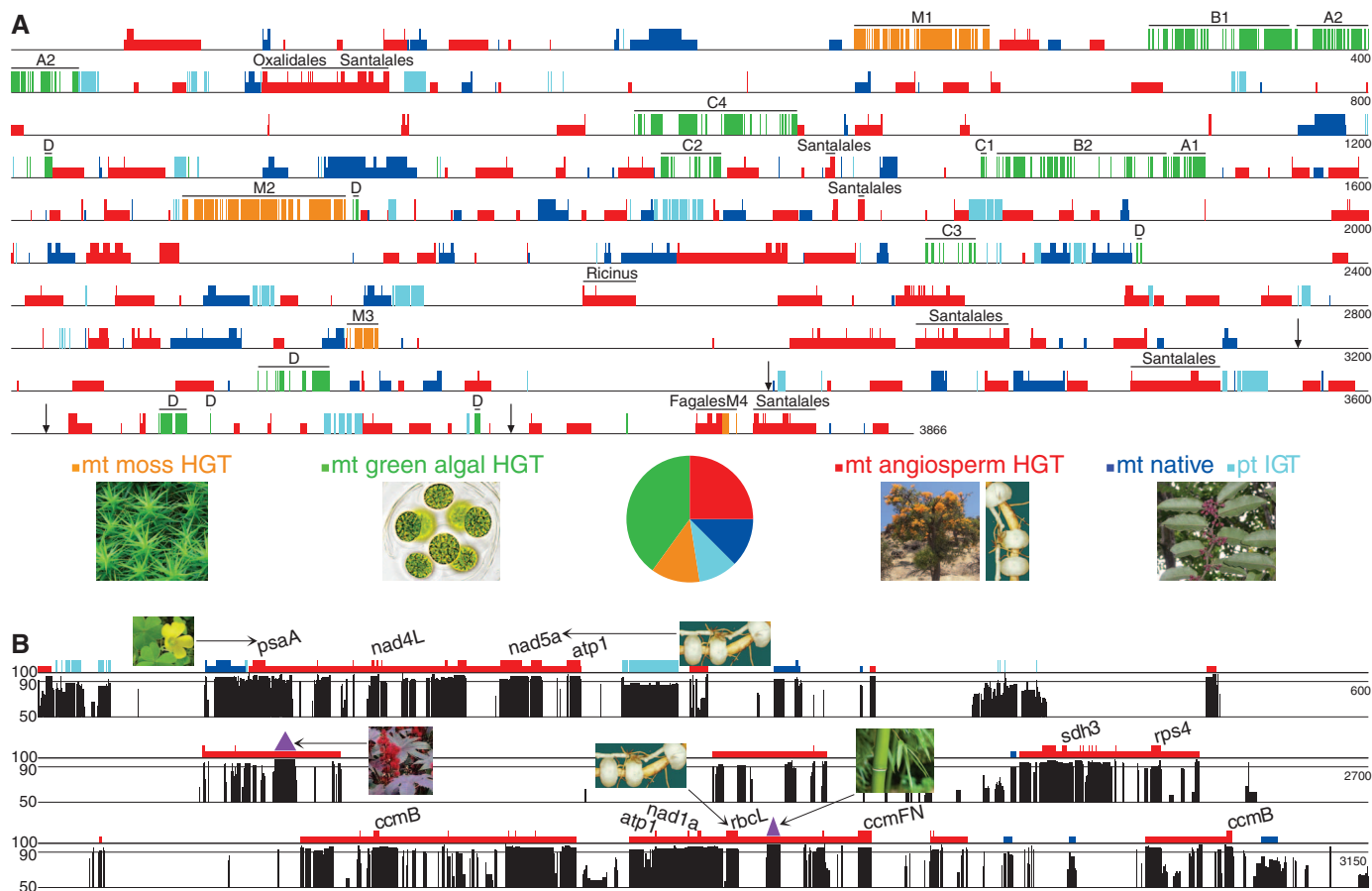


Fig. 1. Foreign DNA in the *Amborella* mitochondrial genome. (A) Map of its five chromosomes shown linearized and abutted (see arrows). Numbers give unified genome coordinates in kb. Shown are regions of inferred organelle origin whose ancestry was assignable (see key: mt, mitochondrial; pt, plastid). Full-height boxes indicate genes. Half-height boxes indicate tracts of native and angiosperm-HGT DNA. Labeled black lines indicate horizontally transferred mitochondrial genomes (Figs. 3 and 4) or partial genomes. M1 to M4 mark a moss-derived genome. A1, A2, B1, B2, and C1 to C4 mark three green algal-derived genomes. D marks the seven fragments of a partial genome from a

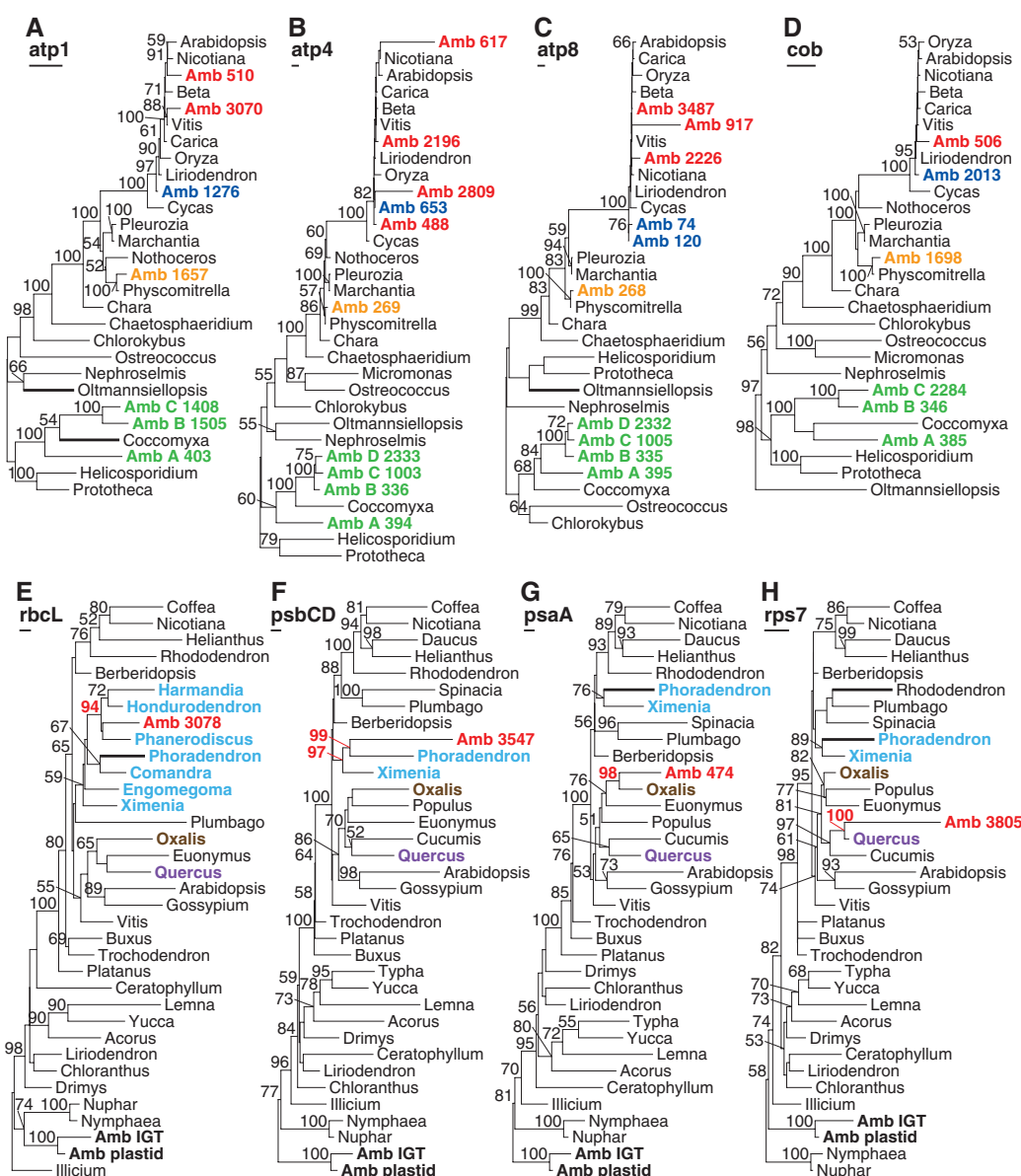
fourth green algal donor. Oxalidales, Santalales, Fagales, and *Ricinus* mark angiosperm tracts whose donors were identified to at least the level of taxonomic order. The pie chart depicts the roughly eight genome equivalents of organelle DNA present in *Amborella* mtDNA. Genome equivalents: mt moss, 1.0; mt green algal, 3.3; mt angiosperm, 2.0; mt native, 1.0; pt IGT, 0.8. See table S11 for photo credits and names of the plants shown. (B) Detailed view of three 150-kb regions of *Amborella* mtDNA. Histograms show the angiosperm score (13). Triangles indicate intergenic regions of species-specific identity to *Ricinus* and *Bambusa* (fig. S21). Gene names are given only for well-supported cases of angiosperm HGT.

These analyses identified 753 kb of DNA as having been acquired from other angiosperms (Fig. 1A and figs. S2 and S4). This DNA contains an average of 2.0 copies of the 32 protein and rRNA genes that are virtually always present in angiosperm mtDNA (table S3) (17) and thus corresponds to roughly two genome equivalents of foreign angiosperm mtDNA. Most (86%) of the 753 kb is intergenic, consistent with the high proportion of intergenic mtDNA in angiosperms (11, 13). About half of the 753 kb shares $\geq 90\%$ sequence identity with one or more sequenced angiosperm mitochondrial genomes (fig. S4). This

One class of plastid-derived DNA played a key role in donor identification. Phylogenetic analysis shows that most of the 138 kb of ptDNA present in *Amborella* mtDNA was acquired through intracellular gene transfer (IGT), that is, from the *Amborella* plastid genome (Fig. 2, E to H, and fig. S19). Analysis of the remaining 10 kb of ptDNA, which probably entered *Amborella* from foreign mitochondria, identified donors with much greater specificity than did the mitochondrial gene analyses (13). Four of the HGT plastid regions identified Fagales, Oxalidales, or the predominantly parasitic Santalales as the donor, while a fifth pointed to Magnoliidae (Fig. 2, E to H, and

Because some angiosperm-HGT tracts in *Amborella* mtDNA are of mixed phylogenetic origin (Fig. 1) (13), some of its foreign DNA may be the product of serial, angiosperm-to-angiosperm-to-angiosperm HGT (13). In particular, the *rbcL* gene of santalalean origin (Fig. 2E) resides only 3 kb from the *Bambusa*-derived sequence on the same 27-kb foreign tract (Fig. 1B). Because all four genes of meaningful length on this tract evidently came from core eudicots (fig. S14), and

(A to D) Mitochondrial gene trees of land plants and green algae reveal diverse donors in *Amborella* mtDNA. Colors are as in Fig. 1. See fig. S8 for outgroups. Bootstrap values $\geq 50\%$ are shown. The number after each *Amb* (*Amborella*) sequence corresponds to its left-most coordinate in kb (figs. S2 and S4). Scale bars correspond to 0.1 [(A) to (D)] or 0.01 [(E) to (H)] substitutions per site. Bold branches are reduced in length by 50%. **(E to H)** Plastid gene trees of angiosperms showing strong support for HGT to the level of taxonomic order: light blue, Santalales [(E) and (F)]; brown, Oxalidales (G); violet, Fagales (H). *Amborella* labels: Amb plastid, gene in *Amborella* plastid; Amb IGT, gene in mitochondrion via IGT; red Amb, gene in mitochondrion via HGT. Outgroups are not shown, but see fig. S19 for more taxon-rich analyses, including outgroups. *rps7* denotes the *rps7-rps12-trnV-rns* cluster.



because parasitic plants are especially active in mitochondrial HGT (5, 7–10), this tract probably came from a santalalean donor that had previously acquired *Bambusa* DNA through HGT (13). The presence of santalalean DNA in six, mostly long HGT tracts (Fig. 1A) suggests that a large portion of the foreign angiosperm DNA in *Amborella* came from Santalales. Indeed, RNA-editing data indicate that the 27-kb tract of putative santalalean origin may actually be part of a much larger (>105 kb) HGT tract (13).

A Graveyard of Foreign Genes

The 197 foreign mitochondrial protein genes in *Amborella* are predominantly pseudogenes, with only 50 (25%) of them having full-length, intact open reading frames (tables S2 and S8). The intact genes are predominantly short (figs. S22 and S23), suggesting that many of these have remained intact by chance; that is, they are pseudogenes that have yet to sustain an obvious pseudogene mutation. Consistent with this, many of these intact genes are not expressed properly.

On the basis of phylogenetic, RNA editing, and/or linkage evidence (table S9) (13), *Amborella* mtDNA is hypothesized to contain a functional, native copy of all but one (*rpl10*) of the 49 mitochondrial protein and rRNA genes inferred to be present in the ancestral angiosperm (fig. S13) (17). cDNA sequencing of 44 of the 48 native genes showed that, with one apparent exception, they are all transcribed and properly RNA edited (table S10) (13). In contrast, no transcripts were detected for many genes of foreign origin, and 13 of 14 tran-

scribed genes of foreign angiosperm origin (eight of them intact) were poorly edited, suggesting that they are pseudogenes (table S10) (13, 19).

The strongest candidates for functional replacement of native genes are tRNA genes. Several native tRNA genes are missing from *Amborella* mtDNA (fig. S13). These, and even some of the native tRNA genes still present (20), may have been functionally replaced by some of its dozens of intact foreign tRNA genes (figs. S2 and S4) (13). This would not be surprising, because cognate tRNAs of diverse origin (plastid, nuclear, bacterial) often replace native tRNAs in plant mitochondrial translation (6, 11, 20, 21). Moreover, even a modest number of tRNA gene replacements could have led to the fixation, through genetic hitchhiking, of a considerable portion of the foreign mtDNA in *Amborella*.

In summary, the great majority of the foreign mitochondrial genes in *Amborella* are unlikely to be functional. Given its six genomes worth of foreign mitochondrial genes, *Amborella* mtDNA serves as a striking example of neutral evolution.

Ancient Transfers, Remarkably Intact

Our ability to date the many mitochondrial HGTs in *Amborella* is limited. However, the extensive pseudogene decay of its foreign DNA (tables S2 and S8)—in conjunction with low mitochondrial substitution rates in angiosperms (including *Amborella*) (fig. S17) (18) and low rates of pseudogene decay (19)—suggests that most transfers are probably millions of years old (13).

Angiosperm mitochondrial genomes typically experience high rates of DNA gain, loss, and

rearrangement (13, 17). *Amborella* mtDNA seems, however, less prone to lose and rearrange DNA. Relative to their many pseudogene mutations, the four moss and green algal whole-genome transfers are surprisingly intact with respect to overall sequence content and arrangement. Only 11% of the protein-coding sequence content inferred to be present at the time of these four transfers has been deleted, mostly due to a few single- or multi-gene deletions (Fig. 4, fig. S6, and tables S2 and S8) (13). The green algal A and B genomes are both intact syntenically except for a single, mutual recombination event, whereas the C and moss genomes have each been fragmented into just four segments (Figs. 1, 3, and 4). In typical angiosperm mtDNAs, comparably old and large tracts of largely nonfunctional DNA would be expected to have mostly been lost by now, and what remained to be more highly rearranged (13, 17).

Mitochondrial Fusion Drives and Limits Mitochondrial HGT

Two mechanisms have been proposed to account for the relatively high frequency of HGT in land plant mitochondria and its absence from plastids of land plants, including *Amborella* (6, 8, 9). First, plant mitochondria are transformation competent (22), whereas no such evidence has been reported for plastids. Second, plant mitochondria regularly fuse in vivo, whereas plastids do not (23, 24). Three aspects of the horizontally acquired DNA in *Amborella* argue that its entry into the mitochondrion was driven principally, if not entirely, by mitochondrial fusion—that is, this DNA entered predominantly in large pieces, including whole genomes (13), is limited to other mitochondrial genomes (13) and is limited to green algae and land plants.

Why are the many *Amborella* donors limited to green plants, as opposed to, for instance, fungi, given their pervasive interactions with plants as mycorrhizal partners, endophytes, epiphytes, and pathogens? We propose that this reflects a phylogenetically deep incompatibility in the mechanism of mitochondrial fusion. The mechanism of mitochondrial fusion in fungi and animals is fundamentally the same, involving a core machinery of dynamin-related guanosine triphosphatases that

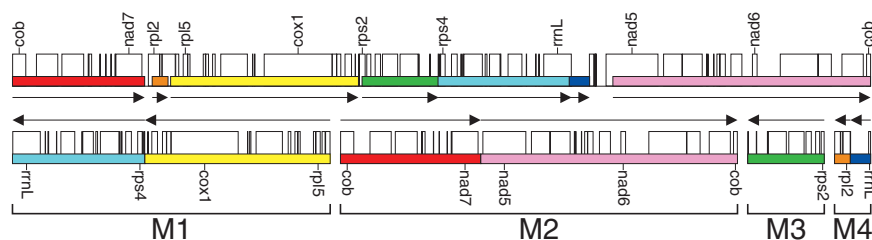


Fig. 3. A nearly full-length moss mitochondrial genome in *Amborella* mtDNA. Colored boxes and arrows indicate the position and relative orientation, respectively, of the seven blocks of synteny between the mitochondrial genome of the moss *Anomodon* (top) and the four moss-derived regions in *Amborella* mtDNA (M1 to M4) (Fig. 1A). Selected genes are shown; see figs. S2 and S6 for all genes.

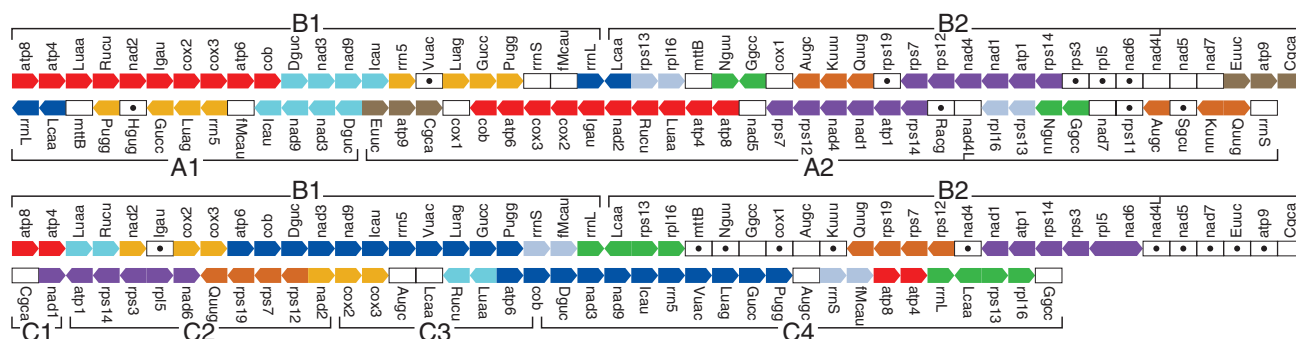


Fig. 4. Pairwise comparisons of the green algal B-genome donor to *Amborella* with the A- and C-genome donors. Brackets on the A, B, and C genomes indicate their fragmentation in *Amborella* (Fig. 1A). Blocks of two or

more genes with identical order in a comparison are colored the same, regardless of gene orientation. Open boxes mark genes present in both genomes but not part of a syntenic block. Bullets mark genes present in only one genome.

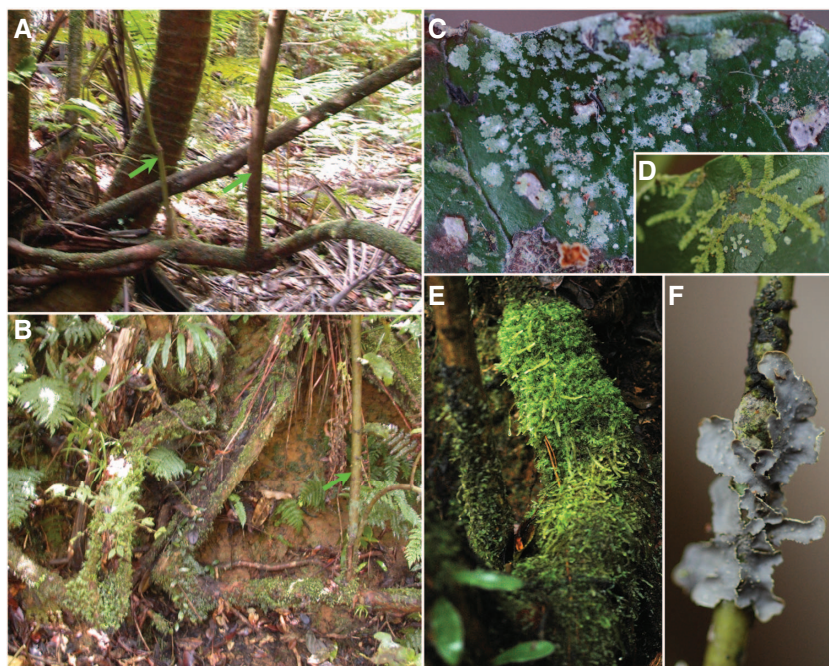


Fig. 5. Ecological setting of HGT in *Amborella*. (A and B) Prostrate branches of *Amborella* with suckers (green arrows) and epiphytes, including mosses, liverworts, ferns, and angiosperms. (C to F) *Amborella* leaves and branches covered predominantly with lichens [(C) and (F)], leafy liverworts (D), and mosses (E). See table S11 for photo credits.

are absent from green plants (25–27). This absence, combined with evidence for differences in the physiological requirements for fusion, has prompted speculation that mitochondrial fusion occurs by a different mechanism in angiosperms than in animals and fungi (24, 27, 28). Our data provide evolutionary support for this hypothesis and also lead us to propose that mitochondrial fusion occurs in a fundamentally similar manner across land plants and green algae (Fig. 6). This model explains why, despite presumably broad phylogenetic exposure to foreign mitochondria, the vast majority of HGT in the mitochondrion of *Amborella*—and other plants (2–10, 13)—is restricted to other plant mitochondria.

Capture of Foreign Mitochondria

Biological vectors large enough to mobilize entire mitochondria, such as pollen (9, 29), insects, and fungi, could account for some of the mitochondrial HGT in *Amborella* (bacteria and viruses are presumably too small to transfer an entire mitochondrion). However, in light of its ecology and development, nonvectored processes involving direct contact between *Amborella* and potential donors probably predominate. *Amborella* grows in montane rainforests, often covered by a diversity of epiphytes, mostly bryophytes (including mosses) and lichens (a potential source of its green algal genomes), and sometimes even other angiosperms (Fig. 5). *Amborella* is often wounded and responds by producing abundant suckers (Figs. 5, A and B). Wounding can break cells belonging to both *Amborella* and the organisms growing on and within it. We postulate

that some of the broken *Amborella* cells are healed and incorporated into a new meristem—a new germline arising thanks to the totipotency of plant cells. Indeed, plant meristems often form in direct response to wounding and may be especially active in “massive mitochondrial fusion” (24). Given the ease of both mitochondrial membrane fusion and mitochondrial genome recombination, those healed cells that have taken up a mitochondrion from another green plant could well incorporate a portion of the foreign mitochondrial genome. A fraction of these transfers could then become fixed.

The wounding-HGT model applies not only to plants that live on *Amborella* but also to parasites. The Santalales—probably the major source of foreign angiosperm mtDNA in *Amborella*—are also the major group of parasitic plants in New Caledonia and the largest group of parasitic angiosperms worldwide (30, 31).

Concluding Remarks

The *Amborella* mitochondrial genome has both captured other mitochondrial genomes whole and retained them in remarkably intact form for ages. Its assemblage of foreign mtDNA probably reflects a range of factors—ecological, developmental, and molecular—that promote the capture of foreign mtDNA and retard its loss and rearrangement. This genome highlights the potential scale of neutral evolution and is thus relevant to current debates on the issue of “junk DNA” in nuclear genomes (32). The greatest importance of this genome is mechanistic: It provides compelling support for mitochondrial fusion as the key

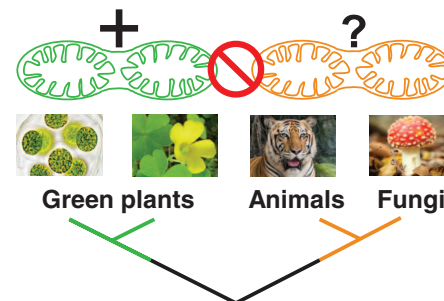


Fig. 6. Evolutionary model of mitochondrial fusion compatibilities. Green and orange indicate different mechanisms of mitochondrial fusion (24, 25, 27, 28), due to either highly divergent evolution from a common ancestral mechanism or independent origins of fusion. See table S11 for photo credits.

that unlocks mitochondrial HGT and for fusion incompatibility as a major barrier to phylogenetically unconstrained mitochondrial “sex.”

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Supplementary Materials

www.sciencemag.org/content/342/6165/1468/suppl/DC1
 Materials and Methods
 Figs. S1 to S23
 Tables S1 to S12
 References (33–76)

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Constraining Exoplanet Mass from Transmission Spectroscopy

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Determination of an exoplanet's mass is a key to understanding its basic properties, including its potential for supporting life. To date, mass constraints for exoplanets are predominantly based on radial velocity (RV) measurements, which are not suited for planets with low masses, large semimajor axes, or those orbiting faint or active stars. Here, we present a method to extract an exoplanet's mass solely from its transmission spectrum. We find good agreement between the mass retrieved for the hot Jupiter HD 189733b from transmission spectroscopy with that from RV measurements. Our method will be able to retrieve the masses of Earth-sized and super-Earth planets using data from future space telescopes that were initially designed for atmospheric characterization.

With more than 900 confirmed exoplanets (1) and more than 2300 planetary candidates known (2), research priorities are moving from planet detection to planet characterization. In this context, a planet's mass is a fundamental parameter because it is connected to a planet's internal and atmospheric structure and it affects basic planetary processes, such as the cooling of a planet, its plate tectonics (3), magnetic field generation, outgassing, and atmospheric escape. Measurement of a planetary mass can in many cases reveal the planet bulk composition, allowing us to determine whether the planet is a gas giant or is rocky and suitable for life as we know it.

Planetary mass is traditionally constrained with the radial velocity (RV) technique using single-purpose dedicated instruments. The RV technique measures the Doppler shift of the stellar spectrum to derive the planet-to-star (minimum) mass ratio as the star orbits the planet-star common center of mass. Although the RV technique has a pioneering history of success laying the foundation of the field of exoplanet detection, it is mainly effective for massive planets around relatively bright and quiet stars. Most transiting planets have host stars that are too faint for precise RV measurements. For sufficiently bright host stars, stellar perturbations may be larger than the

planet's signal, preventing a determination of the planet mass with RV measurements even for hot Jupiters (4). In the long term, the limitation due to the faintness of targets will be reduced with technological improvements. However, host-star per-

turbations may be a fundamental limit that cannot be overcome, meaning that the masses of small planets orbiting quiet stars would remain out of reach. Current alternative mass measurements to RV for transiting planets are based on modulations of planetary-system light curves (5) or transit-timing variations (6). The former works for massive planets on short period orbits and involves detection of both beaming and ellipsoidal modulations (7). The latter relies on gravitational perturbations of a companion on the transiting planet's orbit. This method is most successful for companions that are themselves transiting and in orbital resonance with the planet of interest (8, 9). For unseen companions, the mass of the transiting planet is not constrained, but an upper limit on the mass of the unseen companion can be obtained to within 15 to 50% (10).

Transiting exoplanets are of special interest because the size of a transiting exoplanet can be derived from its transit light curve and combined with its mass, if known, to yield the planet's

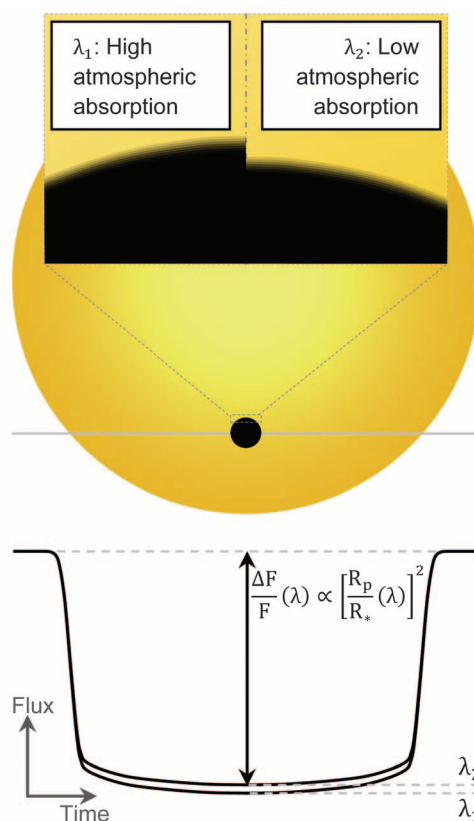


Fig. 1. Transit-depth variations, $\frac{\Delta F}{F}(\lambda)$, induced by the wavelength-dependent opacity of a transiting planet atmosphere. The stellar disk and the planet are not resolved; the flux variation of a point source is observed.

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density, constraining its internal structure and potential habitability. Furthermore, the atmospheric properties of a transiting exoplanet can be retrieved from the host-star light passing through its atmosphere when it transits, but the quality of atmospheric retrieval is reduced if the planet's mass is inadequately constrained (11).

Here, we introduce MassSpec, a method for constraining the mass of transiting exoplanets based solely on transit observations. MassSpec extracts a planet's mass through its influence on the atmospheric scale height. It simultaneously and self-consistently constrains the mass and the atmosphere of an exoplanet, provides independent mass constraints for transiting planets accessible to RV, and allows us to determine the masses of transiting planets for which the RV method fails.

MassSpec: Concept and Feasibility

The mass of a planet affects its transmission spectrum through the pressure profile of its atmosphere [i.e., $p(z)$, where z is the altitude], and hence its atmospheric absorption profile. For an ideal gas atmosphere in hydrostatic equilibrium, the pressure varies with the altitude as $d\ln(p) = -\frac{1}{H}dz$, where H is the atmospheric scale height defined as

$$H = \frac{kT}{\mu g} \quad (1)$$

where k is Boltzmann's constant and T , μ , and g are the local (i.e., altitude dependent) temperature, mean molecular mass, and gravity. By expressing the local gravity in terms of the planet's mass (M_p) and radius (R_p), Eq. 1 can be rewritten as

$$M_p = \frac{kTR_p^2}{\mu GH} \quad (2)$$

Thus, our method conceptually requires constraining the radius of the target as well as its atmospheric temperature, mean molecular mass, and scale height.

A planet transmission spectrum can be seen as a wavelength-dependent drop in the apparent brightness of the host star when the planet transits (Fig. 1). At a wavelength with high atmospheric absorption, λ_1 , the planet appears larger than at a wavelength with lower atmospheric absorption, λ_2 , because of the additional flux drop due to the opaque atmospheric annulus. In particular, a relative flux drop, $\frac{\Delta F}{F}(\lambda)$, is associated with an apparent planet radius, $R_p(\lambda) = \sqrt{\frac{\Delta F}{F}(\lambda)} \times R_*$. Transmission spectroscopy mainly probes low-pressure levels; therefore, the mass encompassed in the sphere of radius $R_p(\lambda)$ (Eq. 2) is a good proxy for the planetary mass. The apparent radius of a planet relates directly to its atmospheric properties due to their effect on its opacity

$$\begin{aligned} \pi R_p^2(\lambda) &= \pi [R_{p,0} + h_{\text{eff}}(\lambda)]^2 \\ &= \int_0^\infty 2\pi r (1 - e^{-\tau(r,\lambda)}) dr \end{aligned} \quad (3)$$

where $R_{p,0}$, $h_{\text{eff}}(\lambda)$, and $e^{-\tau(r,\lambda)}$ are, respectively, a planetary radius of reference—i.e., any radial distance at which the body is optically thick in limbo—looking over all the spectral band of interest, the effective atmosphere height, and the planet's transmittance at radius r (Fig. 2). $\tau(r,\lambda)$ is the slant-path optical depth defined as

$$\begin{aligned} \tau(r,\lambda) &= 2 \int_0^\infty \sum_i n_i(r') \\ &\times \sigma_i[T(r'), p(r'), \lambda] dx \end{aligned} \quad (4)$$

where $r' = \sqrt{r^2 + x^2}$ and $n_i(r')$ and $\sigma_i[T(r'), p(r'), \lambda]$ are the number density and the extinction cross section of the i th atmospheric component at the radial distance r' (12). In other words, a planet's atmospheric properties [$n_i(z)$, $T(z)$, and $p(z)$] are embedded in its transmission spectrum through $\tau(r,\lambda)$ (Eqs. 3 and 4). The integral in Eq. 3 can be split at the radius of reference (because the planet is opaque at all λ at smaller radii), and thus Eq. 3 becomes

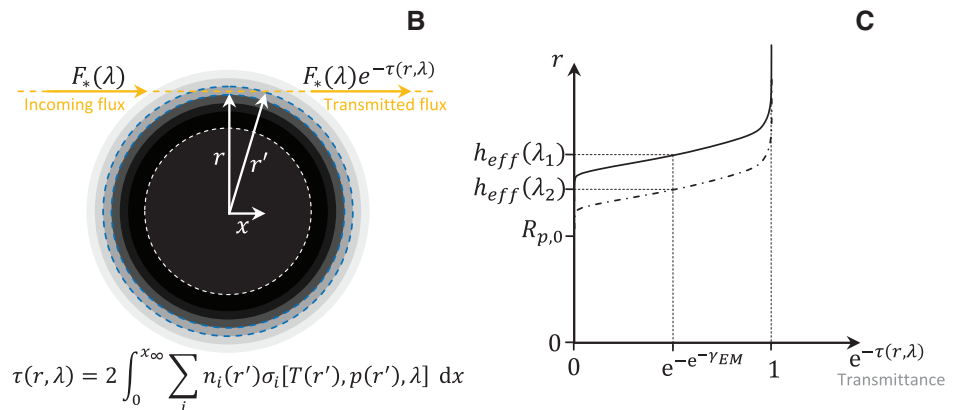


Fig. 2. Basics of a planet's transmission spectrum (planetary atmosphere scaled up to enhance visibility). (A) In-transit geometry as viewed by an observer presenting the areas of the atmospheric annuli affecting the transmission spectrum. (B) Side view showing the flux transmitted through an atmospheric annulus of radius r . (C) Transmittance as a function of the radius

at wavelengths with high and low atmospheric absorption: λ_1 (solid lines) and λ_2 (dash-dotted lines), respectively. Due to higher atmospheric absorption at λ_1 , the planet will appear larger than it does at λ_2 because of the more-extended opaque atmospheric annulus [$h_{\text{eff}}(\lambda_1) > h_{\text{eff}}(\lambda_2)$] that translates into an additional flux drop (14).

$$[R_{p,0} + h_{\text{eff}}(\lambda)]^2 = R_{p,0}^2 + c \quad (5)$$

$$c \approx 2 \int_0^\infty (R_{p,0} + z)(1 - e^{-\tau(z,\lambda)}) dz$$

leading directly to the expression of the effective atmosphere height

$$h_{\text{eff}}(\lambda) = R_{p,0}(-1 + \sqrt{1 + c}) \quad (6)$$

The embedded atmospheric information can be straightforwardly accessed for most optically active wavelength ranges using

$$h_{\text{eff}}(\lambda) = R_{p,0}B(\gamma_{\text{EM}} + \ln A_\lambda) \quad (7)$$

where γ_{EM} is the Euler-Mascheroni constant (13):

$$\gamma_{\text{EM}} = \lim_{n \rightarrow +\infty} \sum_{k=1}^n \frac{1}{k} - \ln n \approx 0.57722 \text{ (supple-}$$

mentary text 1) (14). In the above equation, B is a multiple of the dimensionless scale height and A_λ is an extended slant-path optical depth at the reference radius. The exact formulation of B and A_λ depends on the extinction processes affecting the transmission spectrum at λ (table S1). For Rayleigh scattering

$$B = \frac{H}{R_{p,0}} \text{ and} \quad (8)$$

$$A_\lambda = \sqrt{2\pi R_{p,0} H n_{\text{sc},0} \sigma_{\text{sc}}(\lambda)} \quad (9)$$

where $n_{\text{sc},0}$ and $\sigma_{\text{sc}}(\lambda)$ are the number density at $R_{p,0}$ and the cross-section of the scatterers. Conceptually, Eq. 7 tells us the altitude where the atmosphere becomes transparent for a given slant-path optical depth at a radius of reference, A_λ . For example, if A_λ is 10^4 , then the atmosphere becomes transparent at ≈ 9 scale heights above the reference radius.

Most important, Eq. 7 reveals the dependency of a transmission spectrum on its key parameters: In particular, A_λ is dependent in unique ways on the scale height, the reference pressure, the temperature, and the number densities of the main atmospheric absorbers (supplementary text 1.4), which lead to the mean molecular mass. The uniqueness of these dependencies enables the independent retrieval of each of these key parameters. Therefore, a planet's mass can be constrained uniquely by transmission spectroscopy (Eq. 2).

MassSpec: Applications

Gas Giants

With available instruments, MassSpec is applicable only to hot Jupiters. Their mean molecular mass is known a priori (H/He-dominated atmosphere: $\mu \approx 2.3$), and their temperature is inferred from, for example, emission spectroscopy. Hence, high signal-to-noise ratio (SNR)/resolution transmission spectra are not required to constrain their mean molecular mass and temperature independently. Therefore, the measurement of the Rayleigh-scattering slope in transmission is sufficient to yield the mass of a hot Jupiter because it relates directly to its atmospheric scale height. Because A_λ depends solely on λ through $\sigma_{sc}(\lambda)$ for Rayleigh scattering (Eq. 9), using the scaling law function for the Rayleigh-scattering cross section, $\sigma_{sc}(\lambda) = \sigma_0(\lambda/\lambda_0)^\alpha$, Eq. 7 leads to

$$\alpha H = \frac{dR_p(\lambda)}{d \ln \lambda} \quad (10)$$

with $\alpha = -4$ (15). Therefore, using Eqs. 2 and 10, the planet mass can be derived from

$$M_p = -\frac{4kT[R_p(\lambda)]^2}{\mu G \frac{dR_p(\lambda)}{d \ln \lambda}} \quad (11)$$

Based on estimates (16, 17) of $T \approx 1300$ K, $dR_p(\sim 0.8 \mu\text{m})/d \ln \lambda \approx -920$ km, and $R_p(\sim 0.8 \mu\text{m}) \approx 1.21 R_{\text{Jup}}$ derived from emission and transmission spectra, MassSpec's estimate of HD 189733b's mass is $1.15 M_{\text{Jup}}$. This is in excellent agreement with the mass derived from RV measurements [$1.14 \pm 0.056 M_{\text{Jup}}$ (18)] for this extensively observed Jovian exoplanet. MassSpec's application to gas giants will be particularly important for gas giants whose star's activity prevents a mass measurement with RV [e.g., the hottest known planet, WASP-33b (4)].

Super-Earths and Earth-Sized Planets

The pool of planets accessible to MassSpec will extend down to super-Earths and Earth-sized planets thanks to the high SNR spectra expected from the James Webb Space Telescope (JWST) (launch date 2018) and the Exoplanet Characterisation Observatory (EChO) (European Space Agency M3 mission candidate). We estimate that with data from JWST, MassSpec could yield the mass of mini-Neptunes, super-Earths, and Earth-sized planets up to distances of 500 pc, 100 pc, and 50 pc, respectively, for M9V stars and 200 pc, 40 pc, and 20 pc for M1V stars or stars with earlier spectral types (Fig. 3 and supplementary text 2.4). For EChO, the numbers would be 250 pc, 50 pc, and 13 pc, and 100 pc, 20 pc, and 6 pc, respectively.

In particular, if MassSpec were applied to 200 hours of in-transit observations of super-Earths transiting an M1V star at 15 pc with JWST, it would yield mass measurements with a relative uncertainty of $\sim 2\%$, $\sim 10\%$, and $\sim 15\%$

for hydrogen-, water-, or nitrogen-dominated atmospheres, respectively (Fig. 4 and figs. S9 and S10). The larger significance of the mass measurements obtained for hydrogen-dominated super-Earths results from higher SNR of their transmission spectra, which is due to the larger extent of the atmosphere because of the smaller mean molecular mass of H/He. For the same super-Earths with hydrogen- or water-dominated atmospheres, EChO's data should yield mass measurements with a relative uncertainty of $\sim 3\%$ or $\sim 25\%$ (figs. S11 and S12), respectively; for a nitrogen world in the same configuration, it will not be possible to constrain the mass (fig. S13).

In the future era of 20-m space telescopes, sufficiently high-quality transmission spectra of Earth-sized planets will be available (19). By using MassSpec, such facilities could yield the mass of Earth-sized planets transiting an M1V star (or stars with earlier spectral types) at 15 pc with a relative uncertainty of $\sim 5\%$ (fig. S17). For M9V stars, it would be possible to constrain the mass of Earth-sized planets up to 200 pc, and for M1V stars or stars with earlier spectral types, up to 80 pc (Fig. 3).

Discussion

Finding Habitable Earth-Sized Planets Around Late M Dwarfs in the Next Decade

Late M dwarfs are favorable for any in-transit information, such as transmission spectra, because of their large ratio of radiance over projected area (fig. S19). For that reason, MassSpec can be applied to late M dwarfs more distant than other stars, for a given planet (Fig. 3). If they exist, Earth-sized planets may be detected around late M dwarfs before JWST's launch by SPECULOOS (Search for Habitable Planets Eclipsing Ultra-Cool Stars), a European Research Council mission that will begin observing the coolest M dwarfs in 2016. Their mass will not be constrained by RV because of the faintness of their host stars. However, MassSpec's application to their JWST spectra will yield both their masses and atmospheric properties (Fig. 5), and hence the assessment of their potential habitability.

JWST-EChO Synergy

Time prioritization of JWST and EChO taking into account their synergy would increase the science delivery of both missions. Because the smaller aperture of EChO would enable it to observe brighter stars (i.e., early-type and close-by stars), EChO's and JWST's time could be respectively prioritized on super-Earths and Earth-sized planets for M9V stars closer than 25 pc and for M1V stars (or stars with earlier spectral type) closer than 10 pc (Fig. 3). Similarly, EChO's and JWST's time could be respectively prioritized on gas giants and super-Earths for M9V stars closer than 125 pc and for M1V stars (or stars with earlier spectral type) closer than 50 pc. EChO would be particularly useful to determine the mass and atmospheric

Fig. 3. The boundaries of MassSpec's application domain for 200 hours of in-transit observations. Using JWST, MassSpec could yield the mass of super-Earth and Earth-sized planets up to the distance shown by the black dashed and dotted lines, respectively. Similarly, the maximum distance to Earth for MassSpec's application based on EChO's observations of a mini-Neptune, a super-Earth, and an Earth-sized planet are shown by the blue solid, dashed, and dotted lines, respectively. The green dotted line refers to the case of an Earth-sized planet observed with a 20-m space telescope. The gray area shows the stars too bright for JWST/NIRSpec in the $R = 1000$ mode (J-band magnitude ≤ 7).

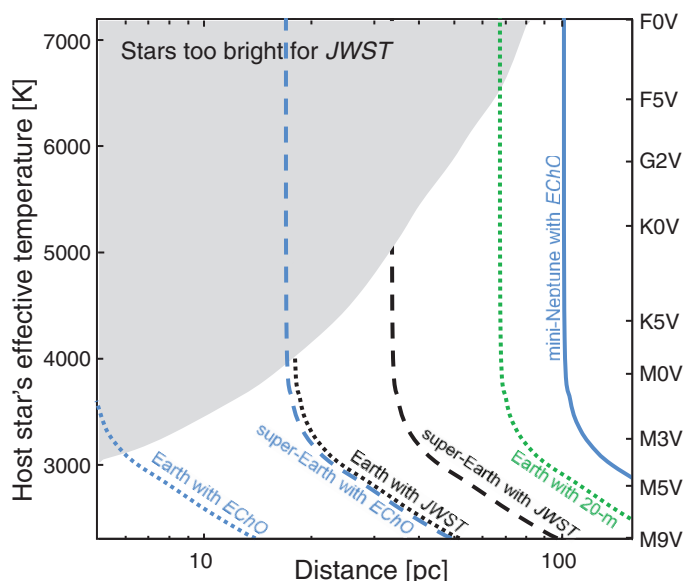


Fig. 4. MassSpec's application to the synthetic transmission spectrum of a water-dominated super-Earth transiting an M1V star at 15 pc as observed with JWST for a total of 200 hours in-transit. (A) Synthetic data and the best fit, together with the individual contributions of the atmospheric species. (B) Normalized posterior probability distribution (PPD) of the atmospheric species number densities at the reference radius. (C) Normalized PPD for the scale height. (D) Normalized PPD for the pressure at the deepest atmospheric level probed by transmission spectroscopy. (E) Normalized PPD for the temperature. (F) Normalized PPD for the exoplanet mass. The diamonds indicate the values of atmospheric parameters used to simulate the input spectrum, and the asterisks in (A) indicate molecules that are not used to simulate the input spectrum. The atmospheric properties (number densities, scale height, and temperature) are retrieved with significance, yielding a mass measurement with a relative uncertainty of $\sim 10\%$.

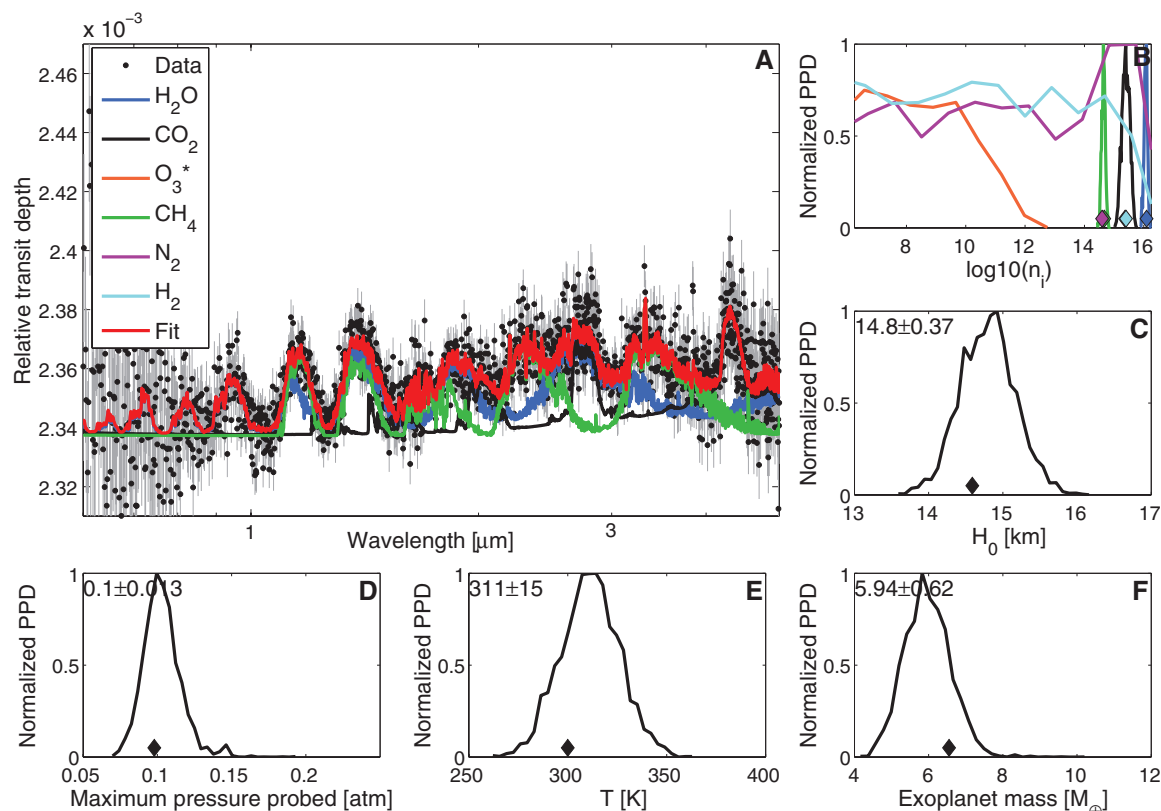
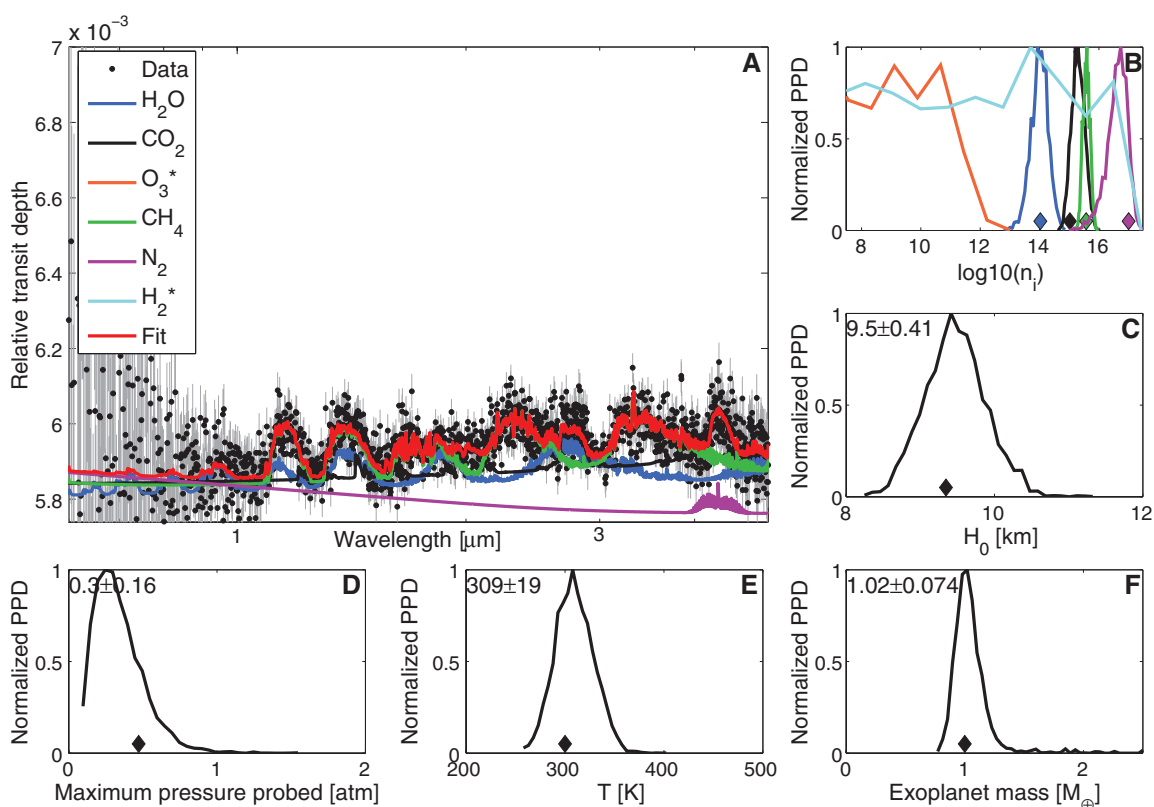


Fig. 5. MassSpec's application to the synthetic transmission spectrum of an Earth-like exoplanet transiting an M7V star at 15 pc as observed with JWST for a total of 200 hours in-transit. (A to F) show the same quantities as on Fig. 4. The atmospheric properties (number densities, scale height, and temperature) are retrieved with significance, yielding a mass measurement with a relative uncertainty of $\sim 8\%$.



properties of gas giants because its wide spectral coverage would allow measurement of their Rayleigh-scattering slope at short wavelengths.

Clouds Will Not Overshadow MassSpec

Clouds are known to be present in exoplanet atmospheres (20) and to affect transmission spectra by limiting the apparent extent of the molecular absorption bands because the atmospheric layers below the cloud deck are not probed by the observations (21). Therefore, the higher the cloud deck, the larger the error bars are with the MassSpec retrieval method due to the reduced amount of atmospheric information available. For example, the uncertainty on the mass estimate of a water-dominated super-Earth with a cloud deck at 1 mbar is twice the uncertainty obtained for the same planet with a cloud-free atmosphere (supplementary text 2.4). However, clouds will not render MassSpec ineffectual because they are not expected for pressures below 1 mbar (22), which is at least three orders of magnitude (i.e., seven scale heights) deeper than the lowest pressure probed by transmission spectroscopy. In other words, there will always be atmospheric information available from transmission spectroscopy.

Complementarity of MassSpec and RV

Transmission spectroscopy is suited for low-density planets and atmospheres and bright or large stars (signal $\propto \rho_p^{-1} \mu^{-1} T_* R_*^{0.5}$), whereas radial velocity measurements are ideal for massive planets and low-mass stars (signal $\propto M_p M_*^{-0.5}$). Therefore, each mass-retrieval method is optimal in a specific region of the planet-star parameter space (supplementary text 3), making the methods complementary.

Possible Insights into Planetary Interiors

Mass and radius are not always sufficient to obtain insights into a planet's interior. MassSpec's simultaneous constraints on a planet's atmosphere and bulk density may help to break this degeneracy, in some cases. A precision on a planet mass of 3 to 15%, combined with the planetary radius, can yield the planetary average density and, hence, bulk composition. Even with a relatively low precision of 10 to 15%, it is possible to infer whether a planet is predominantly rocky or predominantly composed of H/He (23, 24). With a higher planet mass precision, large ranges of planetary compositions can be ruled out for high- and low-mass planets, possibly revealing classes of planets with intermediate density to terrestrial-like or ice or giant planets with no solar system counterpart (25). Typically, the bulk density alone cannot break the planet interior composition degeneracy, especially for planets of intermediate density. However, measurement of atmospheric species may add enough information to reduce some of the planet interior composition degeneracies—for example, the rejection of H/He as the dominant species yields constraint on the bulk composition, independently of the mass uncertainty.

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- We also show that Eq. 7 can be rewritten as $\tau[h_{\text{eff}}(\lambda), \lambda] \approx \tau_{\text{eq}} = e^{-\gamma_{\text{eq}}}$, meaning that the slant-path optical depth at the apparent height is a constant (Fig. 2C). This extends previous numerical observations that $\tau_{\text{eq}} \approx 0.56$ in some cases (15). Therefore, $\tau_{\text{eq}} = \lim_{n \rightarrow +\infty} n \prod_{k=1}^n e^{-1/k} \approx 0.56146$.
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Supplementary Materials

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Figs. S1 to S20
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References (26–39)

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Crystal Structure of a Soluble Cleaved HIV-1 Envelope Trimer

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HIV-1 entry into CD4⁺ target cells is mediated by cleaved envelope glycoprotein (Env) trimers that have been challenging to characterize structurally. Here, we describe the crystal structure at 4.7 angstroms of a soluble, cleaved Env trimer that is stabilized and antigenically near-native (termed the BG505 SOSIP.664 gp140 trimer) in complex with a potent broadly neutralizing antibody, PGT122. The structure shows a prefusion state of gp41, the interaction between the component gp120 and gp41 subunits, and how a close association between the gp120 V1/V2/V3 loops stabilizes the trimer apex around the threefold axis. The complete epitope of PGT122 on the trimer involves gp120 V1, V3, and several surrounding glycans. This trimer structure advances our understanding of how Env functions and is presented to the immune system, and provides a blueprint for structure-based vaccine design.

The envelope glycoprotein (Env) trimer is the only virally encoded antigen on the surface of HIV-1, the pathogen that causes AIDS, and is responsible for viral entry into host cells. The trimer is composed of gp120/gp41 heterodimers and is the target for neutralizing antibodies. Various structures of components of gp120 and gp41, alone and in complex with different ligands, have been determined (1–10). Cryogenic electron microscopy (cryo-EM) and tomography have been integrated with core gp120 x-ray structures to

visualize the Env trimer at resolutions that extend from 30 Å to below 10 Å and, thereby, provide insights into its overall conformation before and after receptor binding (11, 12). However, determining an atomic-level structure of the Env trimer has been difficult. A higher-resolution structure would not only help to explain how the trimer functions during virus-cell fusion, but also guide HIV-1 vaccine design by delineating the key antigenic sites recognized by the humoral immune system and the defenses evolved by the virus as a countermeasure.

During Env synthesis, gp160 precursors trimerize and are subsequently cleaved by proteases of the furin family into gp120 and gp41 subunits, which associate noncovalently before the native complex reaches the surface of infected cells and is then packaged onto virions (13). Cleavage is obligatory for Env trimers to function in viral infection of target cells (14). Virus-cell fusion is

a multistep process involving three major Env conformations, each with distinct roles: (i) prefusion (interacts with CD4 receptor); (ii) extended gp41 intermediate (interacts with CCR5 or CXCR4 co-receptors); and (iii) gp41 six-helix bundle (hemifusion of viral and cell membranes) (15).

The requirement for the cleaved, native Env trimer to undergo conformational changes during receptor binding and fusion makes it metastable, which has substantially hindered both structure determination and vaccine development. The extensive N-linked glycosylation (on average, 81 sites per trimer) creates additional complications for x-ray structural studies. Moreover, membrane-associated forms of Env are more difficult to express and purify in appropriate quantities and qualities than soluble versions. Our approach to these various problems has been to express soluble (that is, truncated before the gp41 transmembrane domain), cleaved forms of trimeric Env (SOSIP gp140) that are engineered to improve their stability and homogeneity. Specifically, a disulfide bond (termed SOS) between gp120 residue 501 (HXB2 numbering) and gp41 residue 605 covalently links these subunits, whereas an Ile-to-Pro change at position 559 (termed IP) strengthens gp41-gp41 associations (16). A re-

cent version of the SOSIP gp140 trimer, based on a tier-2 subtype A virus (BG505) (17), was further engineered to delete all but four residues of the hydrophobic membrane proximal external region (MPER) of gp41 (17–20). Together, these various modifications allow the expression of a thermostable, nonaggregating, and homogeneous soluble Env trimer, BG505 SOSIP.664 gp140, suitable for structural characterization by x-ray crystallography (Fig. 1A). These trimers are reactive with a large panel of diverse broadly neutralizing antibodies (bnAbs), including those to quaternary epitopes, while being minimally reactive with non-neutralizing antibodies that preferentially recognize individual gp120/gp41 subunits and/or uncleaved, non-native trimer forms (17, 18). The near-native antigenic properties of the BG505 SOSIP.664 gp140 trimer suggest that its structure resembles the native viral spike, although we cannot completely rule out slight conformational differences resulting from engineered features, such as truncation of the gp41 MPER and transmembrane domain (19). Here, we show that the BG505 SOSIP.664 gp140 trimers could be successfully crystallized with a highly potent bnAb, PGT122, that targets the glycan-dependent Asn³³² (N332) supersite of vulnerability on gp120 (21).

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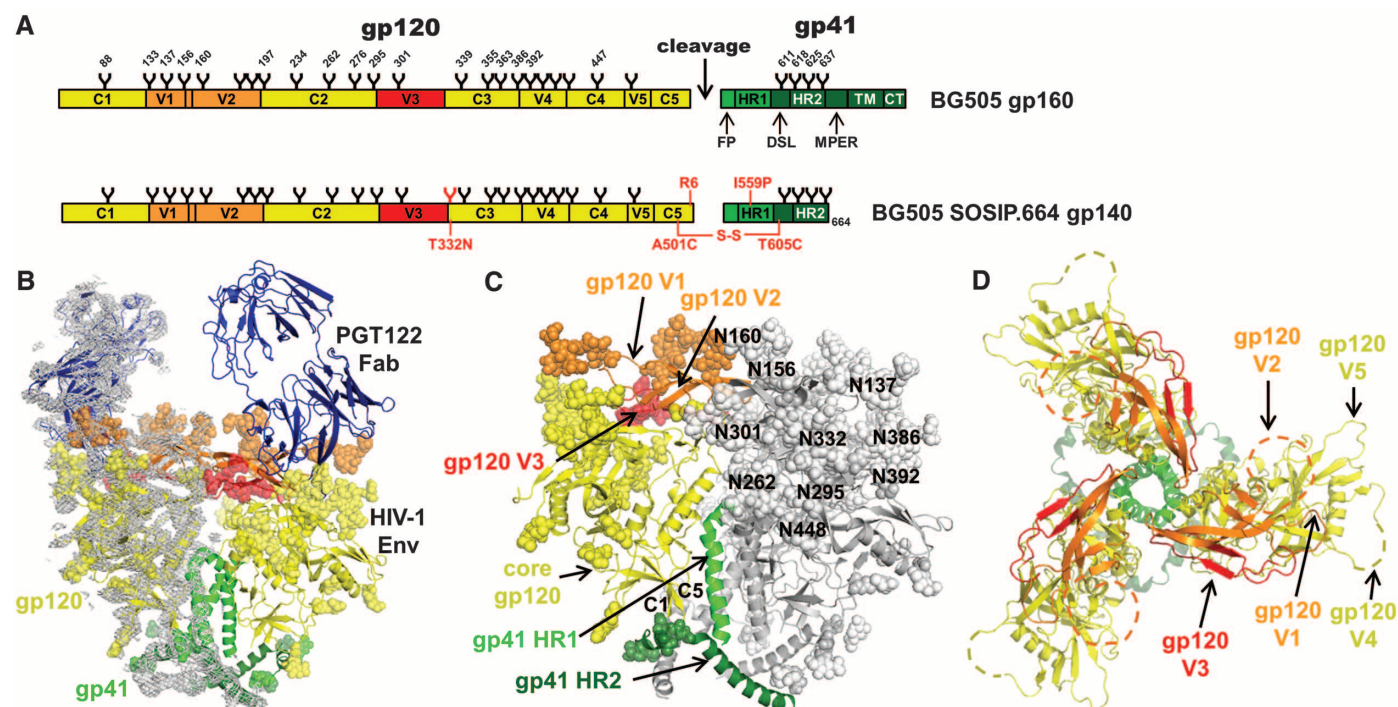


Fig. 1. Overall architecture of a soluble, cleaved, recombinant HIV-1 Env trimer in complex with bnAb PGT 122. (A) Schematic of the HIV-1 Env BG505 SOSIP.664 construct in comparison to full-length gp160. N-linked glycans are shown and numbered on their respective Asn residues. The constant (C1 to C5) and variable (V1 to V5) regions in gp120 and the FP, HR1 and HR2 helices, MPER, transmembrane (TM), and cytoplasmic (CT) elements in gp41 are indicated. The SOSIP mutations are shown in red, as well as the added N332 glycan site. The color coding is shown in (B) to (D). (B) Side view of the soluble Env trimer complex with PGT122 showing two of the three Env gp140 protomers associated with PGT122 Fab (blue). A 2Fo-Fc electron-density map contoured at 1.0 σ is shown as a gray mesh around the leftmost

gp140 protomer. The membrane to which gp41 is attached would be at the bottom of the figure. (C) Side view of the Env trimer. For one of the three protomers on Env, core gp120 is shown in yellow, whereas V1/V2 and V3 regions are highlighted in orange and red, respectively. The main gp41 helical elements are colored in different shades of green. Protein components are rendered according to their secondary structure, and glycans are depicted as spheres. (D) View of Env down the trimer axis. Loops of high variability in gp120 (V1 to V5) all map to the periphery of the trimer and are labeled. Glycans have been omitted for clarity. Dashed lines indicate the location of gp120 V2 and V4 loops for which electron density was absent or ambiguous. The figure was generated with PyMOL (63).

These crystals allowed the structure of an Env trimer to be determined at a resolution of 4.7 Å.

Structure Determination

The BG505 SOSIP.664 construct was expressed in human embryonic kidney 293S GnTI^{-/-} cells, yielding trimers enriched for oligomannose (Man₅-Man₉) glycans [see supplementary materials (22)]. After incubation of the purified trimers with a sixfold molar excess (twofold for the binding sites) of PGT122 Fab, the complex was treated with EndoH glycosidase (fig. S1, A and B) to truncate any accessible N-linked glycans (that is, not buried or occluded by PGT122) to a single *N*-acetyl glucosamine (NAG) moiety that remains covalently attached to the Asn side chain. Crystals of the purified complex diffracted well, albeit anisotropically, to 3.7 Å along the *c* axis, but to lower resolution along the other two axes (fig. S1, C and D). Merging diffraction data from two crystals resulted in a complete data set to a maximum resolution of 4.7 Å (table S1). Phases were obtained by molecular replacement using integrative approaches in which a search model was generated from crystal structures of the unliganded PGT122 Fab variable region [Protein Data Bank identification number (PDB ID): 4JY5 (23)] and CD4-bound gp120 core [PDB ID: 3JWD (5)], docked into our previous ~14 Å EM reconstruction of the same complex [Electron Microscopy Data Bank identification number: 5624 (23)]. Only one complex was present in the asymmetric unit with 82% solvent, which simplified structure determination (fig. S2). Initial phases resulted in a well-defined electron-density map that enabled subsequent placement of the high-resolution PGT122 Fab constant

domains and the gp120 V1/V2 [PDB ID: 3U4E (4)] and V3 [PDB ID: 2ESX (24)] structures. Previously uncharacterized elements in the trimer—such as the gp41 helices, the V1/V2/V3 loops, and various Man₅-Man₉ glycans—were visible in the electron-density maps, as were residual NAG moieties attached to their respective Asn residues (fig. S3). Together, these identifiable features, along with aromatic side chains, aided in model building and refinement at this moderate resolution. In addition, the prominent new features for the Env trimer that we observed in the electron-density maps are the same as those visualized in the accompanying 5.8 Å cryo-EM reconstruction of the same trimer in complex with bnAb PGV04 (fig. S4) (25).

Architecture of the Env Trimer

The soluble BG505 SOSIP.664 trimer adopts a compact mushroom shape reminiscent of the Env trimer prefusion closed conformation determined at 20 Å resolution by EM (11, 26). Three PGT122 Fabs protrude vertically from the membrane-distal gp120 subunits, whereas the gp41 components are membrane-proximal and interspersed with the gp120 C1 and C5 elements (Fig. 1, B to D). Previous high-resolution crystal structures of core gp120 [PDB ID: 3JWD (5)], scaffolded V1/V2 [PDB ID: 3U4E (4)], and the variable domains of PGT122 Fab [PDB ID: 4JY5 (23)] fit well into the electron density of the trimer complex (Figs. 1 and 2 and fig. S5) and have *C*_α root mean square deviations of 1.3, 2.9, and 0.8 Å with the final trimer model, respectively. Thus, the core gp120 elements in the trimer adopt conformations similar to those observed in unliganded gp120 (2) or gp120 in complex with various ligands [spe-

cifically CD4 (3, 5), b12 (9), VRC01 (8), PGT128 (7), PGT135 (1), and PG9/PG16 (4, 6)]. In addition, all previously described disulfide bonds in gp120 (3) are present, and all of the variable loops (V1 to V5) are on the outside of the structure (Fig. 1D).

Our Env trimer structure contrasts markedly with one recently described for an uncleaved, membrane-bound JR-FL Env trimer (27). Compared with our soluble cleaved structure, the uncleaved trimer EM structure differs substantially even in the gp120 core, as well as in the arrangement of the gp41 helices. Our structure also does not contain the large hole that has been reported to be present in the uncleaved Env trimer (27–29). It remains to be determined whether these differences are attributable to the use of different forms of trimer (soluble, truncated, cleaved versus detergent-solubilized, almost full-length, uncleaved) or reflect concerns about the cryo-EM methodology used to derive the uncleaved trimer structure (30–34).

Comparison with Component Crystal Structures

High-resolution crystal structures of monomeric gp120 have largely been obtained with the use of a core construct stabilized in the CD4-bound conformation and with the V1/V2/V3 elements truncated (1, 3, 8–10). We observe only a few small deviations from these gp120 crystal structures, mostly in elements leading to and from V1/V2 (Fig. 2A and fig. S6). The differences are expected because V1/V2 undergoes major conformational changes when gp120 binds CD4 (2, 11, 12). In the trimer structure, clear density

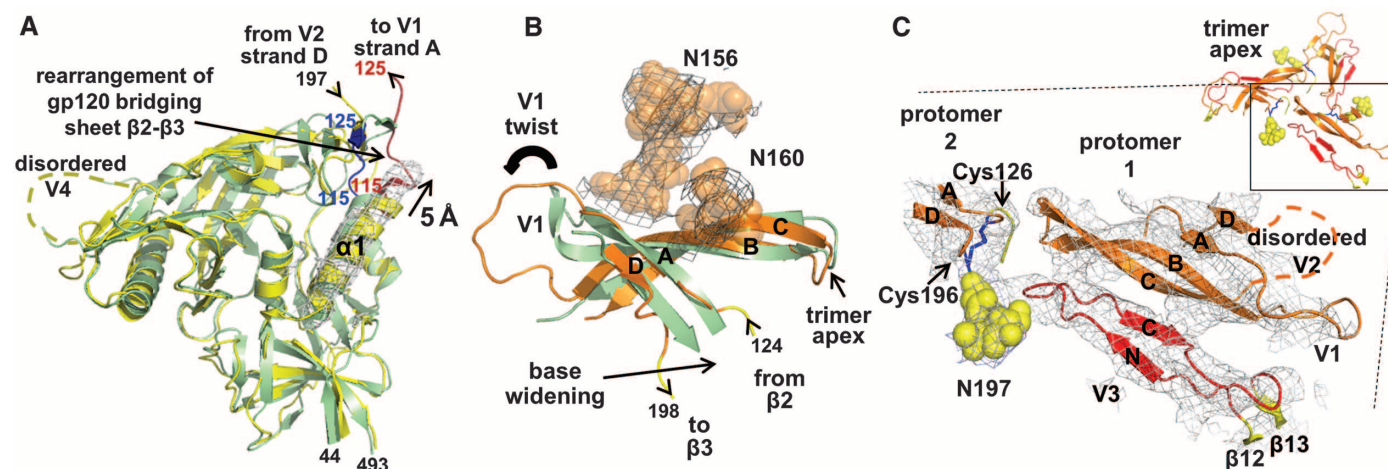


Fig. 2. Comparison of gp120 and components, as observed in high-resolution crystal structures and in the soluble HIV-1 Env trimer. (A) High-resolution crystal structure of core gp120 [PDB ID: 3JWD (5), pale green] superimposed on the gp120 component of the soluble, cleaved SOSIP.664 trimer crystal structure (yellow). A longer α 1 helix (gp120 residues 99 to 117) likely contributes to rearrangement in the bridging sheet, particularly in β 2 and β 3, which extend into V1/V2 atop the trimer. The gp120 β 2-proximal residues 115 to 125 are highlighted in blue and brown in core gp120 and trimeric gp140, respectively. A 2Fo-Fc electron-density map contoured at 1.0 σ is shown as a gray mesh around the α 1 helix.

(B) Superimposition of the scaffolded gp120 V1/V2 crystal structure (PDB ID: 3U4E, pale green) on V1/V2 in the trimer crystal structure (orange). There are differences in V1 and in the β 2 and β 3 connecting strands. Electron density for carbohydrates at gp120 N156 and N160 is shown as a 2Fo-Fc gray mesh contoured at 1.0 σ . (C) Structural arrangement of gp120 V1/V2 (orange) and V3 (red) in the context of the trimer. A 2Fo-Fc electron-density map contoured at 1.0 σ is shown as a gray mesh around V1/V2 and V3 elements. All structures are depicted according to secondary structure elements, with glycans depicted as yellow spheres and the Cys¹²⁶-Cys¹⁹⁶ disulfide bond shown in blue. The figure was generated with PyMOL (63).

for an additional helical turn (residues 114 to 117) is observed in the gp120 α 1 helix (residues 99 to 113), which leads into V1/V2 (Fig. 2A). In addition, the β 2 (119 to 123) and β 3 (199 to 201) strands are only partially involved in forming the bridging sheet with β 20 and β 21; instead, compared with gp120 monomeric cores, β 2 and β 3 flip and translocate slightly toward the trimer apex to connect with strands A and D of V1/V2, respectively (Fig. 2A). This elongated α 1 helix-bridging sheet arrangement is also observed in the accompanying cryo-EM structure of the SOSIP gp140 trimer in complex with bnAb PGV04 (25). Whether this β -strand inversion at the V1/V2 base in the trimer structures—compared with the CD4-bound (3, 5), antibody-bound (8–10, 35), and unliganded core monomeric crystal structures (2)—can be attributed to oligomer-monomer differences, changes induced by CD4-binding, or the truncation of V1/V2 in core gp120 is not yet known.

Electron density at the trimer apex indicates that V1/V2 adopts a four-stranded Greek-key β -sheet arrangement similar to that observed in the high-resolution crystal structures of V1/V2 scaffolds in complex with bnAbs PG9 [(4), Fig. 2B)] and PG16 (6). The V1 loop (residues 132 to 140) connecting strands A and B adopts a more flat-

tened structure that is parallel to the four-stranded β -sheet topology, differing slightly from its more vertical conformation in the V1/V2 scaffolded structure (4) and in the accompanying high-resolution cryo-EM trimer structure in complex with a receptor binding site antibody (25). It is unclear whether this difference in V1 orientation is a consequence of PGT122 binding or arises because V1 is a flexible loop. In the trimer crystal structure, the Cys¹²⁶-Thr¹²⁸ and Leu¹⁹³-Cys¹⁹⁶ segments diverge from one another, while still maintaining the Cys¹²⁶-Cys¹⁹⁶ disulfide bond, and these segments no longer contribute to V1/V2 strands A and D (Fig. 2B). Instead, they interact atop the Env spike with V1/V2 strands B and C and the conserved crown of the V3 loop from a neighboring protomer (Fig. 2C). We observe no clear electron density for V2 loop residues 178 to 190, which were also disordered in the V1/V2 scaffolded structures (4, 6).

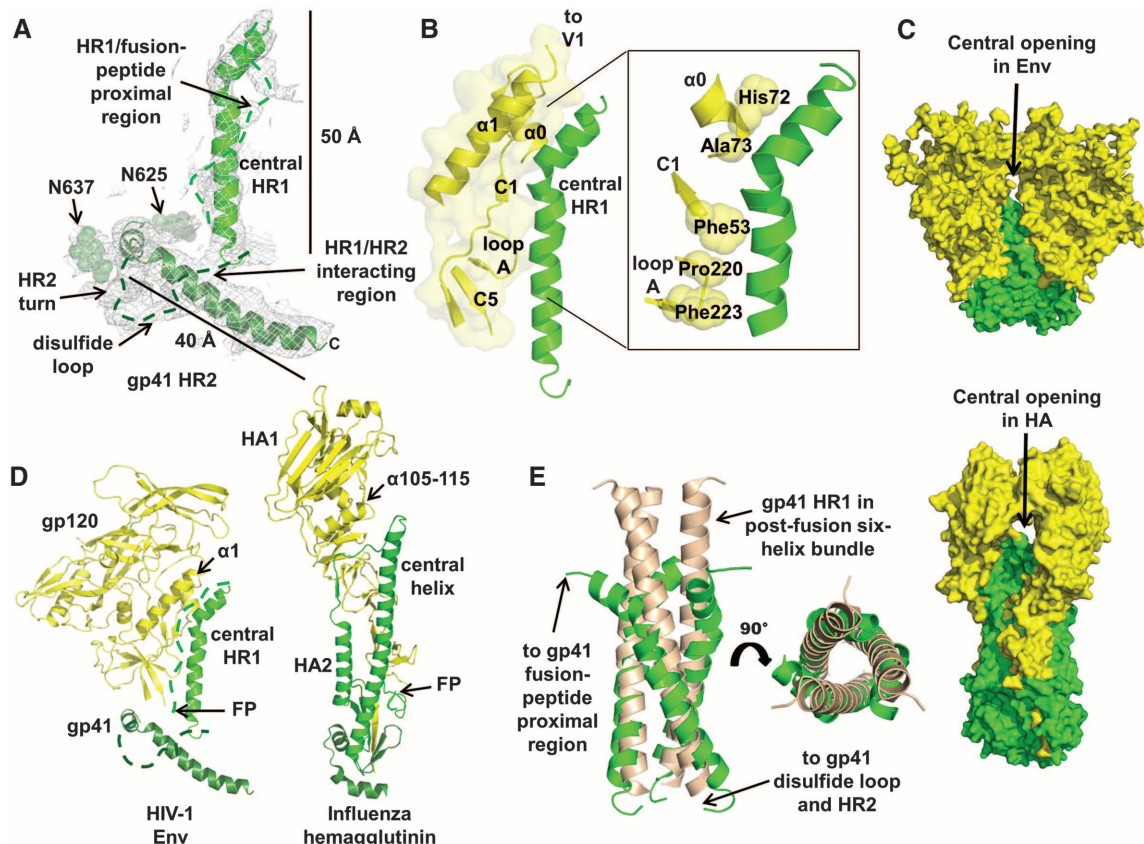
The gp120 V3 loop completes the trimer apex and forms a β -hairpin with its two antiparallel β strands nestling directly below V1/V2 strands B and C of the same protomer (Fig. 2C). V3 peptides complexed with Abs adopt similar β -hairpin structures (24, 36, 37), as does the V3 region of monomeric gp120 (38) (fig. S5). The V3 crown is buried

under a NAG moiety from the N197 glycan at the C-terminal end of V2 strand D from an adjacent protomer (Fig. 2C). Removing this glycan from other viruses increases nAb sensitivity (39) and can confer CD4-independent entry into CCR5-expressing cells (40). Our structure suggests that the N197 glycan helps to stabilize native Env by occluding V3 and inhibiting its premature release before CD4 binding. Any heterogeneity in the presence or composition of the N197 glycan may contribute to the reactivity of non-neutralizing V3 antibodies with BG505 SOSIP.664 trimers in some binding assays (17). Overall, the trimer structure is compatible with both intra- and intertrimeric (cis-trans) V3 shielding by V1/V2 (41, 42).

gp41 Architecture

Strong electron density for helices attributable to gp41 is clearly visible in the trimer structure (Fig. 3A). Three central helices (six turns) extend $\sim$ 30 Å along the trimer axis, perpendicular to the viral membrane, and are ascribed to gp41 heptad-repeat 1 (HR1). Similar helices were recently observed in an $\sim$ 9 Å cryo-EM reconstruction of the KNH1144 SOSIP gp140 trimer (12). An additional short helix (two and a half turns) extends from the central helix bundle but is kinked away from the trimer axis.

Fig. 3. Structural organization of gp41 in the soluble cleaved HIV-1 Env trimer. (A) Overall arrangement of gp41 elements from one protomer is shown in a gray 2Fo-Fc electron-density map contoured at 1.0 σ . Carbohydrates are shown as spheres. Dashed lines delineate connecting electron density for which a chain trace and secondary structure determination was ambiguous at this moderate resolution. (B) Regions of contact between the gp120 inner domain and the gp41 central helix. The inset shows hydrophobic residues in the gp120 high-resolution crystal structure from C1, α 0, and loop A that line the interface with gp41 HR1. (C) A surface rendering of two of the three HIV-1 Env and influenza hemagglutinin (HA) (PDB ID: 4FNK) protomers (back protomer omitted) emphasizes the similarity in position and size of a small, central interprotomer opening that presumably facilitates conformational changes during the fusion process. (D) Comparison between the structures of HIV-1 Env and influenza HA (PDB ID: 4FNK), the prototype type I fusion protein. There are notable similarities in the position of structural elements in the two glycoproteins. (E) The trimeric arrangement of gp41 HR1 in



the postfusion conformation [PDB ID: 2X7R (50), beige] superimposes closely with the central HR1 in the soluble HIV-1 Env trimer, which is similar to the retention of the three-helix bundle at the top of the long HA2 helix in influenza HA. All structures are depicted according to secondary structure elements. The figure was generated with PyMOL (63).

These two HR1 helices pack against hydrophobic gp120 residues in C1 and C5, as well as loop A (Fig. 3B), and are capped by the gp120 $\alpha 1$ helix (Fig. 3B). Overall, these topological features are consistent with previous mutagenesis and structural studies that suggest gp41-gp120 interactions may propagate long-range effects upon receptor binding (5, 43).

The weak and diffuse electron density corresponding to the fusion peptide (FP) and the residues that connect it to the HR1 central helix makes model building of this region particularly difficult at this resolution (fig. S7A), but this is likely indicative of a lack of regular secondary structure (44). Nonetheless, residues adjacent to the N termini of the HR1 helices initially wrap around the gp120 $\alpha 0$ helix, forming a metastable loop-helix structure akin to similar elements in influenza HA2 (45) (Fig. 3, C and D) that lead to an extension of the central helix in the post-fusion form (46). Overall, the central trimeric coiled-coil arrangement of the fusion protein (gp41) surrounded by three receptor subunits (gp120) is a characteristic of type-1 membrane-fusion proteins (47–49). We now extend that hallmark to the capping of the central helices by another helix ($\alpha 1$ and $\alpha 105$ –115) in both gp120 and HA. A small interprotomer central opening is created at the top of the central helix in both fusion proteins (Fig. 3C), as well as in Ebola and PIV5/RSV fusion proteins, that presumably facilitates conformational rearrangements of the head domains (e.g., gp120, HA1) during the fusion process. The Env trimer structure also agrees well with what is known about the transition from a pre- to postfusion conformation, in that the three HR1 central helices adopt a similar arrangement in the postfusion six-helix bundle (Fig. 3E) (50).

A second well-defined helical element that is ~ 40 Å long at an $\sim 60^\circ$ angle relative to the HR1

central helix is located at the bottom of the trimer, proximal to the membrane, where it wraps around the trimer base (Fig. 3A). We interpret this long helix (seven turns) as the C-terminal portion of the HR2 sequence, which is consistent with a cryo-EM reconstruction in the accompanying paper (25), where deletion of residues 651 to 664 from the BG505 SOSIP.664 trimer eliminated the density corresponding to the end of this helix (25, 51). Strong electron density between the bottom of HR1 and the middle of the HR2 helix is likely indicative of substantial intrasubunit gp41-stabilizing interactions. The residues that are N-terminal to the long HR2 helix appear to adopt a horseshoe conformation for the polypeptide backbone (Fig. 3A and fig. S4B); the NAG moieties at glycosylation sites 625 and 637 are clearly visible in the electron-density maps and define the location of turns. Strong electron density, predominantly in a flat-extended shape characteristic of β strands, is present between the C terminus of HR1 and the N terminus of HR2, where the gp41 disulfide loop [(DSL), residues 585 to 609] is thought to be located (fig. S7B). However, we cannot determine the arrangement of these gp41 components with confidence at this resolution, and a further complication is the close proximity of gp120 C1 and C5 elements that most likely participate in tertiary structure interactions with residues connecting HR1 to HR2, including the DSL (5, 52). As such, we attribute the approximate location of the gp41 DSL and, hence, the engineered intersubunit disulfide bond to the base of the Env trimer. Finally, no conclusion can be drawn about the arrangement of the gp41 MPER because it is not present in the BG505 SOSIP.664 gp140 trimer (19, 20). The gp41 HR2 may continue to extend in the same orientation as it enters the MPER, or it could change direction near residue

664 (53, 54) so that the MPER could align parallel to the trimer axis.

Structural Definition of the PGT122 Epitope

The PGT121 family of antibodies potentially neutralizes $\sim 70\%$ of circulating HIV-1 viruses (21, 55), and PGT121 is highly effective in protecting against mucosal challenge in a macaque model of HIV-1 infection (56). The crystal structure presented here agrees well with our lower-resolution negative-stain EM reconstruction of the same PGT122-trimer complex (23) (fig. S8) but now allows a more complete description of the epitope. As initially predicted (21), the N332 Man_{8/9} glycan that is a central feature of the PGT122 epitope sits directly at the junction between the light-chain complementarity-determining region 2 (LCDR2) and the 26-residue heavy-chain CDR3 (HCDR3) (Fig. 4A and fig. S9). Residues in the LCDR1, LCDR3 and light-chain framework 3 (LFR3) regions of PGT122, which were predicted by alanine-scanning mutagenesis to be important for HIV-1 neutralization (23), interact directly with the gp120 V3 base near Ile³²³-Arg³²⁷, a key part of the epitope (21, 57). The trimer structure also helps explain why PGT121-class bnAbs depend on V1/V2 residues for locking gp120 into the pre-CD4 conformation (23); in fact, gp120 V1 residues 135 to 139, including the glycan at N137, come into close proximity to HCDR1, HCDR2, HCDR3, and LCDR3 of PGT122 (Fig. 4A and fig. S9). The N137 oligomannose glycan in the trimer structure superimposes almost perfectly with the biantennary complex glycan that occupies the groove formed by the PGT121 heavy-chain CDRs in previous crystal structures (23, 55, 58) (Fig. 4, A and B). Alanine scanning mutagenesis of gp120 glycans clearly indicates that bnAbs of the PGT121 family

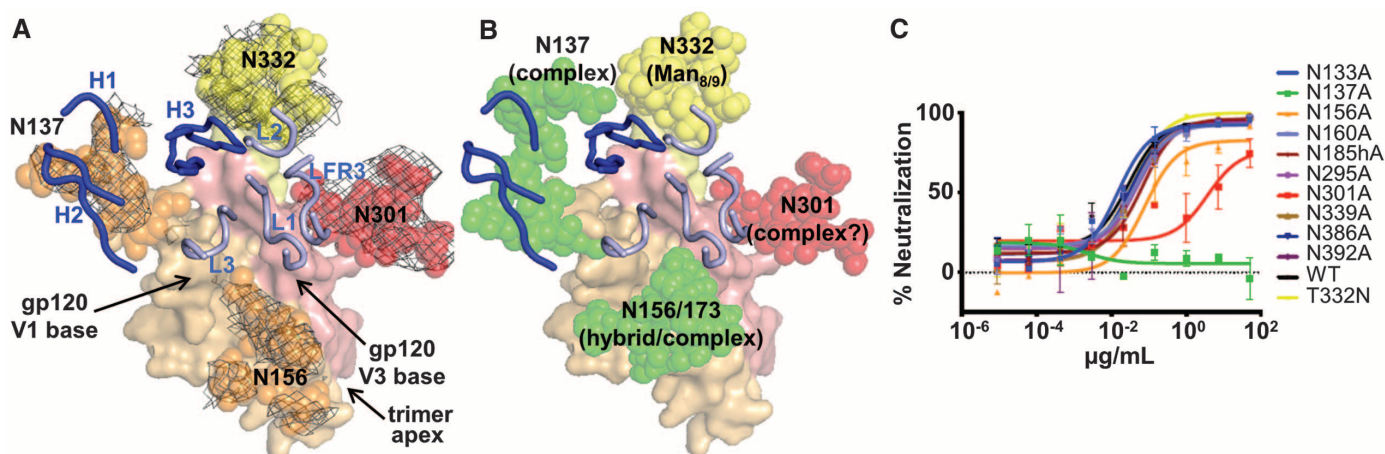


Fig. 4. Complete structural definition of the PGT122 epitope. (A) In addition to the N332 glycan (yellow), the PGT122 bnAb recognizes both protein and glycan elements near the base of gp120 V1 (orange) and V3 (red) to mediate broad and potent HIV-1 neutralization. Heavy- and light-chain CDRs are shown as dark and light blue tubes, respectively. Electron density for oligomannose glycans (spheres) surrounding the PGT122 epitope is shown as a 2Fo-Fc gray mesh contoured at 1.0σ . (B) Superimposition of the PGT122 epitope with glycans from PGT121 [PDB ID: 4JY4 and 4FQC (23, 55)] and PG16 [PDB ID: 4DQO (6)]

liganded crystal structures. PGT122 binding is compatible with the involvement of complex or hybrid glycans at gp120 N137 and N156/173 (green) when the trimer is expressed in human cells capable of making this type of glycan. The figure was generated with PyMOL (63). (C) The use of glycan knockout mutants of HIV-1 BG505 pseudoviruses reveals the importance of glycans at N137, N156, and N301 for PGT122 recognition and neutralization. These glycans are part of the PGT122 epitope in the trimer crystal structure to varying extents. A, Ala; T, Thr; WT, wild type. Error bars indicate SEM obtained from duplicate measurements.

depend on N137 for neutralizing the BG505 virus (Fig. 4C and fig. S10). Locating this key residue in the crystal structure has allowed us not only to delineate the full PGT122 epitope, but also to confidently determine the relative position of V1/V2 elements at the trimer apex. In addition to N137 and N332, the N156 and N301 glycans are also protected from EndoH deglycosylation by PGT122. Although the composition and nature of the glycoforms on native Env are still uncertain, PGT122 recognition is compatible with the presence of a complex or hybrid carbohydrate at position N156/N173, as observed in the crystal structure of a V1/V2 scaffold with bnAb PG16 (6) (Fig. 4B). The N156 and N301 glycans do have a clear involvement, albeit a minor one, in neutralization by PGT121-family bnAbs (Fig. 4C and fig. S10). Thus, the Env trimer structure reveals the complexity of the PGT122 epitope, which involves the V1 and V3 base and four glycans (N137, N156/N173, N301, and N332).

Revisiting Glycan-Dependent bnAb Epitopes in the Context of the HIV-1 Env Trimer

The recent isolation (21, 59) and structural characterization (1, 4, 6, 7) of potent glycan-reactive

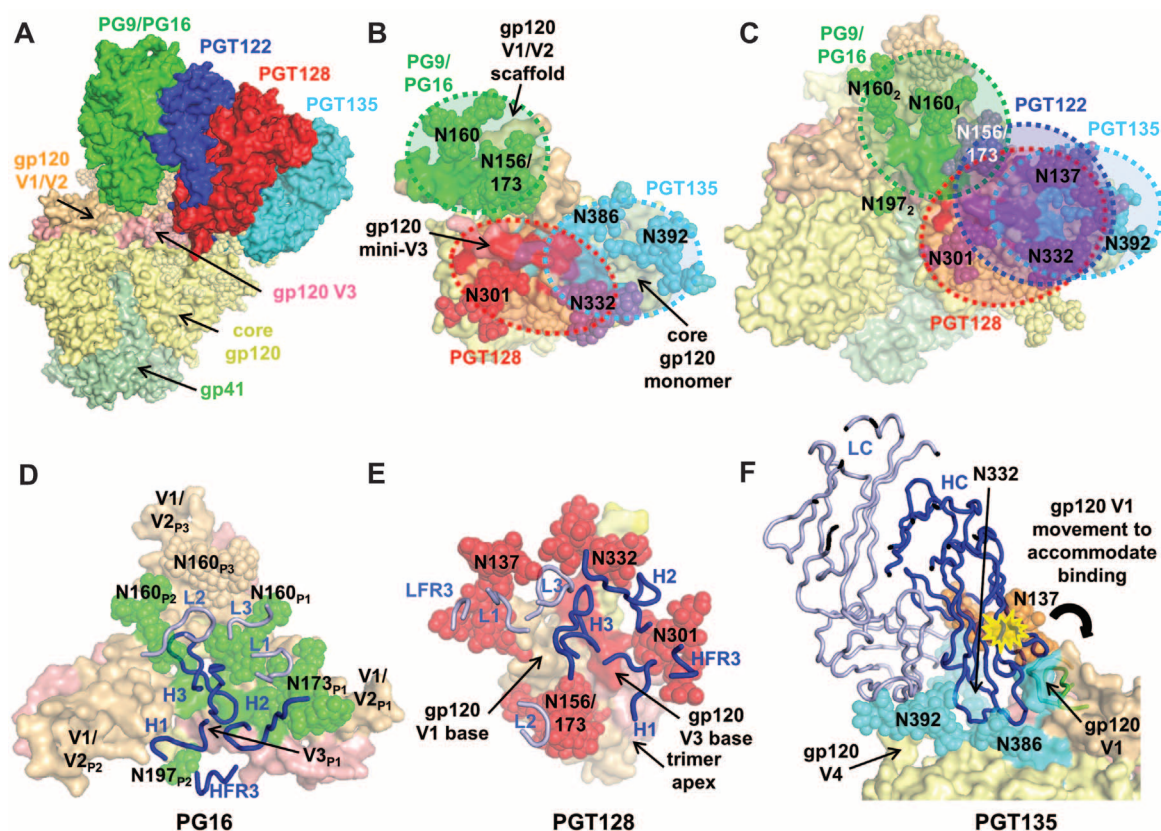
bnAbs has emphasized the need to precisely define the composition of various glycans that are sites of vulnerability on Env. The trimer structure allows us to reevaluate previous crystal structures of glycan-dependent bnAbs that were obtained with monomeric core gp120 or epitope scaffolds. When viewed in the context of the trimer, the gp120 epitopes for the N160-dependent (PG9/PG16) and N332-dependent (PGT122, PGT128, and PGT135) epitopes are in close proximity (Fig. 5A). Once considered separate sites of vulnerability (Fig. 5B), these two epitope clusters are larger than were first thought and overlap to some extent, helping to explain why some of these antibodies compete with one another in certain binding assays (21) (Fig. 5C). All of these glycan-dependent bnAbs use their six CDR loops, in addition to framework regions, when recognizing their epitopes. As previously suggested (18), PG9 and PG16 would interact with two N160 glycans across different protomers in the trimer apex (Fig. 5D). In addition, whereas one side of the elongated HCDR3 hammerhead inserts into the trimer apex, the other is available to interact with elements of V3 (Fig. 5D) and may help explain why V3 residues influence neutralization by

PG16 (59). The trimer crystal structure also suggests that HCDR1 and HFR3 of PG9/PG16 may interact with the V3-protecting N197 glycan on an adjacent protomer (Fig. 5D). PGT128 would also make contacts with Env elements additional to those previously reported in the crystal structure with a gp120 outer domain lacking V1/V2 (7) (Fig. 5E). Thus, LCDR1, LCDR2, and LFR3 of PGT128, for which no previous role could be assigned, are now predicted to be in close proximity to V1 elements, including N137 and N156 (60), whereas a change in the V1 loop orientation is required to allow PGT135 binding (Fig. 5F). Indeed, a V1 orientation closer to that observed in the V1/V2 scaffold or in the high-resolution cryo-EM trimer structure (25) would remove a clash with N137 and facilitate PGT135 interaction with V1. A flexible role for V1 might help to explain the plasticity of PGT135-Env interactions seen in previous EM studies (1).

Conclusion

The crystal structure of the soluble BG505 SOSIP.664 trimer is in excellent agreement (fig. S4) with the accompanying cryo-EM structure of the same Env construct as a complex with PGV04

Fig. 5. Revisiting glycan-dependent epitopes of bnAbs in the context of the soluble Env trimer structure. (A) Superimposition of the cocystal structures of PG16 (green), PGT128 (red), and PGT135 (light blue) on the PGT122 (dark blue)-Env trimer crystal structure reveals that these glycan-dependent epitopes overlap. (B) Cocystal structures of glycan-dependent bnAbs in complex with monomeric gp120 or scaffolded gp120 elements provide crucial, but incomplete details of their epitopes and, hence, how they recognize Env. The various bnAb epitopes are colored individually on the surfaces, with overlapping elements in purple. (C) Superimposition of the PG9/PG16, PGT128, and PGT135 cocystal structures on the soluble trimer-PGT122 cocystal structure reveals that these glycan-dependent bnAbs have expanded epitopes, which creates overlapping sites of vulnerability on HIV-1 Env. (D) Model of the expanded PG16 epitope on Env. Antibody interactions additional to those previously described in the PG9/PG16-V1/V2-scaffold cocystal structures (4, 6) are predicted to occur with gp120 V3, as well as with residues N160 and N197 of the adjacent protomer. Protomers are denoted with P1, P2, and P3 subscripts, and the positions of the hypervariable loops 1 and 2 (attached to the V1/V2 framework) are denoted by V1/V2. The view is slightly offset from looking down the trimer axis. (E). Model of the expanded



PGT128 epitope includes additional interactions with glycans at N137 and N156 mediated via the PGT128 light chain (light blue). (F) PGT135 would clash (yellow star) with the gp120 V1 conformation recognized by PGT122, based on superimposition of the PGT135-core gp120 cocystal structure (1). A slight reorientation of gp120 V1 (green) would allow PGT135 to interact, consistent with the hypothesis that the V1 loop is flexible enough to permit different modes of bnAb interaction. Heavy and light chains are shown as dark and light blue tubes, respectively. The figure was generated with PyMOL (63).

Fab (25). Structures determined by these two independent techniques show complete concordance in the major new features that are visualized at the present level of 5 to 6 Å resolution (fig. S4). We have defined the overall architecture of the soluble trimer, as well as the secondary, tertiary, and quaternary interactions between gp120 and gp41 that are involved in its assembly. The trimer is relatively tightly packed, especially in gp41, but with a small opening between the Env apex and the top of the central gp41 helices. The gp120 subunits are held together, at least in part, by association of the V1/V2/V3 regions at the apex of the trimer (11, 61, 62). The gp41 central helices provide the main stabilizing contact between gp41 and gp120. Finally, the complete definition of how neutralization epitopes are presented in the context of the trimer, here and in the accompanying manuscript (25), should help the design of next-generation immunogens as candidate vaccines.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S10
Tables S1 and S2
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Cryo-EM Structure of a Fully Glycosylated Soluble Cleaved HIV-1 Envelope Trimer

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The HIV-1 envelope glycoprotein (Env) trimer contains the receptor binding sites and membrane fusion machinery that introduce the viral genome into the host cell. As the only target for broadly neutralizing antibodies (bnAbs), Env is a focus for rational vaccine design. We present a cryo-electron microscopy reconstruction and structural model of a cleaved, soluble Env trimer (termed BG505 SOSIP.664 gp140) in complex with a CD4 binding site (CD4bs) bnAb, PGV04, at 5.8 angstrom resolution. The structure reveals the spatial arrangement of Env components, including the V1/V2, V3, HR1, and HR2 domains, as well as shielding glycans. The structure also provides insights into trimer assembly, gp120-gp41 interactions, and the CD4bs epitope cluster for bnAbs, which covers a more extensive area and defines a more complex site of vulnerability than previously described.

HIV-1 currently infects more than 34 million people worldwide and causes AIDS. The availability of antiviral therapies has greatly reduced the death toll, particularly in the Western world, but has not yet reduced the global spread of this deadly pathogen. A successful preventive vaccine would be a large step toward this critical goal. The trimeric viral envelope glycoprotein (Env) spike, a major vaccine development target (1), consists of three gp120 subunits that contain the CD4 receptor and co-receptor binding sites and three gp41 subunits that drive membrane fusion. Immune selection pressure creates extensive Env sequence variation that complicates vaccine development, but trimer-targeting broadly neutralizing antibodies (bnAbs) provide important clues about vulnerable Env sites (1). Critical features of bnAb epitopes have been revealed by x-ray structures of fragment antigen binding (Fab) complexes with the gp120 core, gp120 outer domain, gp41 peptides, and scaffolded epitopes, or from glycan arrays (2–9). These structures are based on only a subcomponent of the Env spike and do not reveal the full complement of intersubunit contacts and constraints. Low-resolution electron microscopy (EM) structures

of the trimer provide an overall architecture (10–16) but do not define the molecular details of bnAb epitopes.

Here, we used cryo-EM to study soluble (truncated at residue 664), cleaved recombinant trimers from the BG505 genotype, stabilized by specific substitutions, including a disulfide bond (termed SOS) between residue 501 (HXB2 numbering) and 605, and an Ile-to-Pro mutation at position 559 (termed IP) (17, 18). These BG505 SOSIP.664 gp140 trimers are highly stable and homogeneous, have a near-native antigenicity profile (19), and display a well-defined shape when viewed by negative-stain EM at intermediate resolution (11, 12, 14, 20). We present here the cryo-EM structure at 5.8 Å resolution of this Env trimer in complex with bnAb PGV04 against a CD4 binding site (CD4bs) epitope. The structure reveals the overall organization of Env, the interaction between gp120 and gp41 subunits, and how trimer formation affects the CD4bs and its associated bnAb epitopes.

Specimen Preparation, EM Data Acquisition, and Image Processing of SOSIP Trimers

We produced BG505 SOSIP.664 gp140 trimers in human embryonic kidney (HEK) 293T cells, and therefore they have a typical human cell glycosylation profile. The Env trimer is relatively small by EM standards (~425 kD, of which almost half is glycan) and lacks features that facilitate high-resolution image processing (21). We therefore adopted a cryo-EM feature enhancement strategy, as recently described (22), by adding PGV04 Fabs as fiducial markers for computational alignment of the trimer. We recorded the EM data on a direct electron detector, which improves the signal relative to conventional methods and enables correction for beam-induced motion and specimen drift (23). Image-processing algorithms similar to those that have recently provided near-atomic resolution characterization

of select macromolecular complexes (24, 25) were used in the analysis. Together, these cryo-EM technical advances, combined with design and production of a stable soluble Env trimer, enabled reconstruction of the SOSIP.664-PGV04 complex to 5.8 Å resolution (Fig. 1 and fig. S1). The reconstructed electron potential map provided sufficient detail for modeling most of gp120, including the variable loops and the heptad repeat 1 (HR1) and HR2 components of gp41 (movie S1). The EM reconstruction was validated by the Fab and gp120 densities that were in excellent agreement with the previously determined structures, by several recently described quantitative metrics for EM (21, 26, 27), and also by an independently obtained x-ray structure of the same trimer (from HEK 293S GnTI[−] cells and with a simpler glycan profile) in complex with the PGT122 bnAb at a similar resolution (fig. S2H) (28). The EM map presented here is substantially improved in resolution and in features relative to previous trimer reconstructions; it also revealed partially discontinuous density surrounding the periphery of the trimer that is consistent with N-linked glycans on both gp120 and gp41 (fig. S4) (29).

Structural Arrangement of gp120 and Variable Loops V1, V2, and V3

The gp120 core crystal structure in complex with PGV04 [PDB ID: 3SE9 (30)] was docked into the EM map and further refined (31). The crystal structure of a scaffolded V1/V2 protein [PDB ID: 3U4E (9)] could also be fitted into density at the trimer apex (Fig. 2A and fig. S5A). Densities corresponding to *N*-acetylglucosamine moieties of the glycans at the Asn¹⁵⁶ and Asn¹⁶⁰ sites (numbering relative to the reference strain HXB2) within V1/V2 are apparent at the trimer apex (Fig. 2B and figs. S4 and S5A) and are consistent with the arrangement of the V1/V2 Asn¹⁵⁶ and Asn¹⁶⁰ glycans predicted by a low-resolution model of the same trimer in complex with bnAb PG9 (14). The V1 loop is in a slightly different conformation from that seen in scaffolded CAP45 V1/V2 (9). The V2 loop could not be built in its entirety but was localized above the CD4bs (fig. S5, A and B) in a position that restricts access to the epitope. The V3 loop is situated directly beneath the V1/V2 hairpin with the tip pointing toward the trimer axis and interacting with the V2 base from the adjacent protomer, in a different configuration than previously observed (Fig. 2C and fig. S5, A and C) (8). The Asn¹⁹⁷ glycan at the V2 base is at the interprotomer interface (Fig. 2B and fig. S5A) and may shield V3 epitopes on the trimer; various V3 antibodies have higher affinities for monomeric than for trimeric Env, consistent with such steric constraints (32, 33). Overall, the gp120 subunits have a compact globular configuration and are assembled into a trimer through intra- and interprotomer interactions at the apex that involve the V1/V2 and V3 loop structures (Fig. 1 and Fig. 2B), consistent with previous observations (34, 35). This arrangement exposes several basic residues at the trimer

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apex that are important targets for bnAbs such as PG9, PG16, PGT145, and CH01, which have acquired negatively charged sulfated tyrosine moieties in their paratopes (9) (Fig. 2D).

Structural Arrangement of gp41

gp41 forms a pedestal at the base of the trimer (Fig. 3, A and B). Each protomer is characterized by two long, prominent helices. The first (HR1) forms a three-helix bundle with the neighboring protomers in the trimer core; the second (HR2) wraps around the outer periphery of the trimer base, angling downward and with its C terminus proximal to the viral membrane (Fig. 3, A and B). The three-helix bundle is formed by the C-terminal half of HR1 and resembles the arrangement in the crystal structure of postfusion gp41 (36), and also in a proposed open, intermediate state as observed by single-particle EM (37) (Fig. 3, C and D). To assess whether the three-helix bundle is a feature induced by PGV04 binding, we calculated an independent 12.7 Å reconstruction of the unliganded trimer (fig. S6). The three-helix bundle was present in this unliganded structure; hence, it is likely that the gp41 conformation

that we describe here represents the closed, pre-fusion state, and not an activated intermediate as previously suggested (37). Just above the three-helix bundle and below the trimer apex, we observe a small opening that is continuous with the exterior (fig. S7). The large hole that is a reported feature of the trimer core in some previous models is likely a result of lower-resolution reconstructions (>20 Å) (38), as illustrated in fig. S3.

To identify the gp41 C terminus, we calculated an ~ 8 Å reconstruction of a similar trimer but with the last 14 amino acids deleted (designated SOSIP.650) (Fig. 3E). Difference maps showed that a segment corresponding to ~ 3.5 helical turns, or ~ 14 amino acids, was absent from the SOSIP.650 reconstruction (Fig. 3E and fig. S8). Additionally, our data indicate that residues around position 650 in HR2 interact strongly with the bottom of HR1 (fig. S9).

The N-terminal half of HR1, including a short helix, and the fusion peptide proximal region (FPPR) and fusion peptide (FP) extend from the top of the internal coiled coil around the three-fold axis and turn down toward the trimer base

(Fig. 3A). The internal region at the base of the trimer contains additional density that could not be assigned unambiguously but likely corresponds to the intervening residues between HR1 and HR2, as well as the gp120 C1 and C5 regions (Fig. 3, A and B). This interpretation is consistent with the known intersubunit interactions between gp120 C1/C5 and gp41 residues ~ 589 to 610 (18). This element of gp41 contains the internal disulfide-bonded loop and also the engineered Cys substitutions that covalently link gp120 residue 501 to gp41 residue 605 via a disulfide bond in SOSIP gp140 (18). The relatively hydrophobic gp120 N and C termini and nearby gp41 residues are sequestered toward the middle of the trimer. We have tentatively assigned the hydrophobic FP to this solvent-inaccessible region (Fig. 3B); there are similarities here to other viral fusion glycoproteins from influenza, respiratory syncytial virus, and Ebola virus (28, 39–41). Localization at the gp120-gp41 interface would allow the FP to be released in response to CD4 and co-receptor-induced conformational changes within gp120 and gp41 that drive membrane fusion.

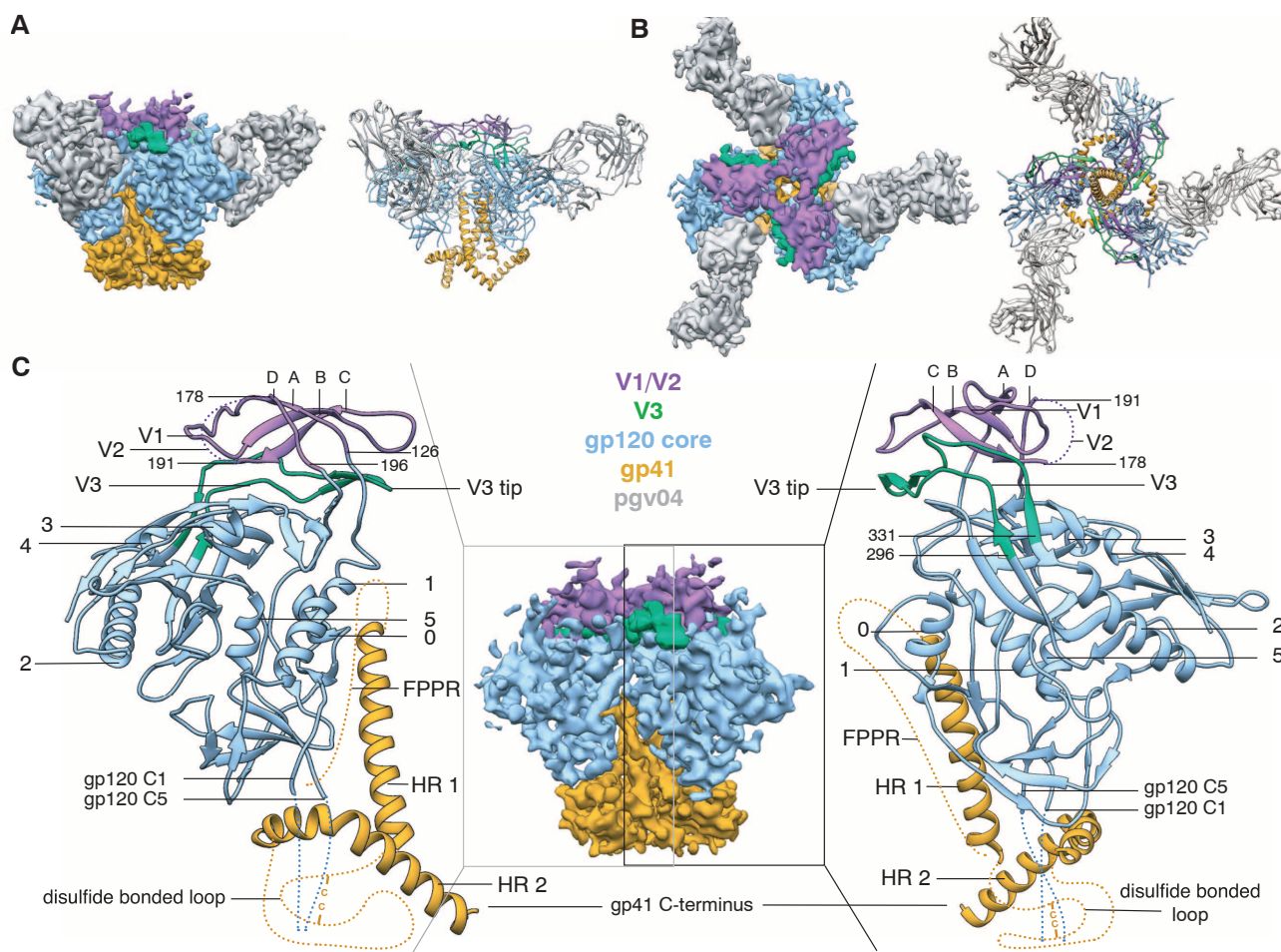


Fig. 1. 5.8 Å EM reconstruction and model of Env trimer in complex with PGV04. (A and B) Side (A) and top (B) views of BG505 SOSIP trimer EM reconstruction (left) and corresponding model (right). Segmentation and color coding: gray, PGV04; blue, gp120; orange, gp41; purple, V1/V2;

green, V3. (C) The center panel shows a side view of the EM map along with the Fab density removed. The outer panels show the modeled portion corresponding to the boxed region in the middle panel. The viral membrane would be at the bottom of the figure.

PGV04 Binding Site on the Env Trimer

Subtle differences in the Fab PGV04 interaction with gp120 are apparent when the monomeric and trimeric forms of core gp120 are aligned, which may be explained by trimer-specific contacts (Fig. 4A). On the glycosylated trimer, the Fab light chain interacts extensively with glycan Asn²⁷⁶ (Fig. 4B and fig. S17). The same glycan prevents the VRC01 germline antibody from binding to gp120 monomers (42), so it may be a general impediment to this bNAb class. The glycosylated trimer EM structure also contains additional density for a region corresponding to the V2 loop and/or glycans (Fig. 4B) that likely play a role in recognition of the CD4bs.

Many SOSIP.664 trimer particles had fewer than three PGV04 Fabs bound, even though the Fab was in molar excess of trimer during sample preparation. We used template-based 3D sorting of single-particle images to generate trimer populations with three, two, one, and zero Fabs bound. Each of the subsets was then independently refined to generate maps with resolutions of 5.8 Å, 7.9 Å, 16.9 Å, and 19.1 Å, respectively (figs. S10 to S15). The 7.9 Å, 2-Fab reconstruction (Fig. 4C) sufficed to interpret subtle structural differences created by the presence or absence of a bound Fab. Specifically, PGV04 binding induced positive density corresponding to the putative V2 loop and/or glycans Asn³⁶³ and Asn³⁸⁶ (Fig. 4D). Additional density corresponding to glycan Asn³⁰¹

from the neighboring gp120 protomer and Asn²⁷⁶ from the same gp120 protomer was also seen to interact with PGV04 (fig. S16C) (43). One interpretation is that PGV04 binding stabilizes both the glycans and the flexible loops in the trimer structure.

Solution isothermal titration calorimetry (ITC) measurements also revealed that PGV04 bound BG505 SOSIP.664 trimers in a substoichiometric manner (<2), consistent with the EM results (Fig. 4E). The EM map does not reveal an obvious structural impediment to stoichiometric PGV04 binding (i.e., 3 per trimer). One hypothesis for why some trimers do not bind the bNAb, or do so at a low stoichiometry, is that incomplete or differential glycan processing creates some microheterogeneity. PGV04 binding to 293S cell-produced trimers that bear only high-mannose sugars was again substoichiometric (i.e., <2); however, when trimers were enzymatically deglycosylated, the number of bound Fabs increased from <2 to ~2.5 and the average binding affinity K_D improved to 73 nM from 135 nM (Fig. 4F), which suggests that specific glycans of variable composition and size can indeed interfere with antibody binding to the CD4bs. The unfavorable entropic contribution of the binding signal was also lower for the deglycosylated trimers. The entropic cost of locking normally flexible glycans in place may be yet another viral defense against antibodies, potentially one that restricts the stimulation of bNAb germline

B cell receptors (42, 44). Eliminating such glycans may facilitate the design of immunogens intended to induce CD4bs antibodies (42, 44).

Quaternary Nature of the CD4 Binding Site

The CD4bs is relatively conserved and immunogenic in the context of HIV-1 infection. Env subunit vaccines, such as monomeric gp120 and uncleaved gp140, have failed to elicit bnAbs against this site while often inducing non-neutralizing or poorly neutralizing Abs that recognize overlapping epitopes (1). This is likely because the CD4bs is presented in a non-native context and thus elicits antibodies that are non-neutralizing because they cannot bind to compact native trimers. The EM structure that we describe delineates the narrow range of approach angles available to CD4bs bnAbs when engaging the trimer, and shows how epitope complexity makes it difficult to target this site effectively via non-native immunogens.

The CD4-induced subdomain known as the bridging sheet is presented on the SOSIP.664 gp140 trimers differently from how it appears on various versions of monomeric gp120 (4). Specifically, the strand arrangement at the base of V1/V2 is reversed on the trimer, such that V2 forms an adjacent/parallel interaction with β 20 instead of the antiparallel β -interaction between the V1 base and β 20 found in the gp120 core (Fig. 5A and fig. S18). Thus, quaternary inter-

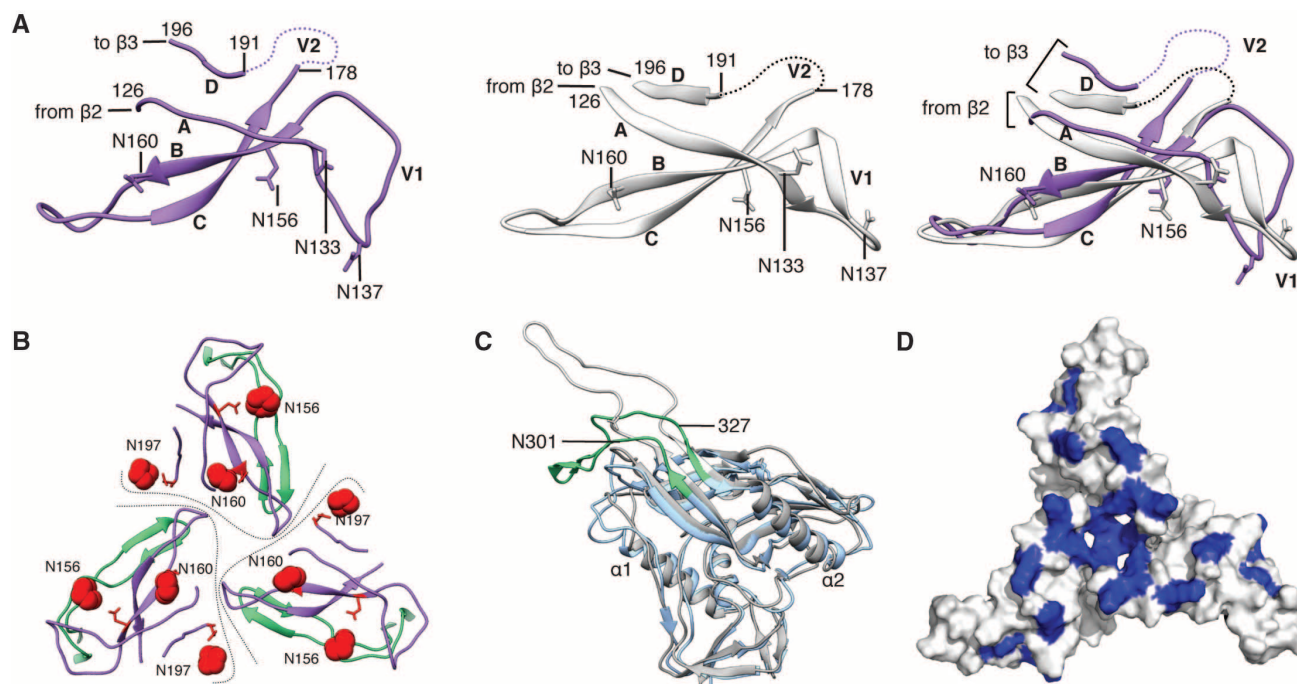


Fig. 2. Structures of gp120 V1/V2/V3 variable loops. (A) Comparison of the V1/V2 domain from the EM model (purple) and the previously published x-ray structure (3U4E, white) with key residues labeled (N, Asn). The structures are nearly identical except for the base of V1/V2 (strands A and D) and the V1 loop. (B) Quaternary arrangement of V1, V2, and V3 regions of gp120 at the top of the trimer. The positions of glycans at Asn¹⁵⁶, Asn¹⁶⁰, and Asn¹⁹⁷ have been highlighted as red spheres. Lines denote approximate protomer boundaries. (C) Superposition of gp120 and the V3 loop from the EM model (blue/green)

onto a previous x-ray structure containing a V3 loop (2B4C, gray/white). Whereas gp120 is structurally conserved, the V3 β -hairpin loop exhibits a different configuration. Specifically, V3 bends around a hinge formed near residues 302 and 326. The N-terminal residues of V3 up to residue 301 and the C-terminal residues after residue 327 are structurally similar. Residues 1 to 90 and 126 to 196 are omitted for clarity. (D) Surface view of the modeled portion in (B), with the basic residues (Arg and Lys) colored blue. A large number of basic amino acids are localized to the trimer apex.

actions mean that the topological relationship between the base of V2 (which includes the Asn¹⁹⁷ glycan) and the CD4bs is not the same on trimeric and monomeric gp120.

The neighboring protomer also plays an important role in restricting access to the CD4bs. When the heavy chain of PGV04 or the related VRC01 bnAb binds the trimer, it is within 5 Å of a loop (residues 61 and 62) that precedes a short α helix ($\alpha 0$) in C1 of a neighboring gp120 protomer (Fig. 5B); likewise, when CD4 (PDB ID: 4JM2) is docked into the CD4bs on the trimer, it also contacts an adjacent protomer (Fig. 5B). The $\alpha 0$

helix is directly downstream from His⁶⁶, a residue implicated in regulating the sampling of the CD4-bound conformation (45). Thus, the first stage of CD4 binding may require the trimer to flex to an extent, but once initial contact is made, the trimer is further destabilized, and co-receptor binding and fusion can proceed.

Docking CD4bs antibodies with a range of neutralization capabilities into the EM trimer model suggests that the crystal structures of monomeric gp120-Ab complexes do not completely define this epitope cluster. Additional, quaternary structure-dependent contacts are important for

CD4bs Fabs to recognize trimeric Env, with the V1/V2 loop structure influencing how they bind and hence neutralize (Fig. 5C). Previous mutagenesis data are now more readily interpretable; for example, the light-chain CDR1 and CDR3 residues are essential for neutralization by the b12 bnAb (46) because they are positioned to interact with V1/V2 (fig. S19). The non-neutralizing Fabs b13 (PDB ID : 3IDX) and F105 (PDB ID: 3HI1) (6) clash with V1/V2 (same protomer) and V3 (adjacent protomer), impeding their ability to bind the trimer (Fig. 5C). These results are consistent with both BG505 virus neutralization

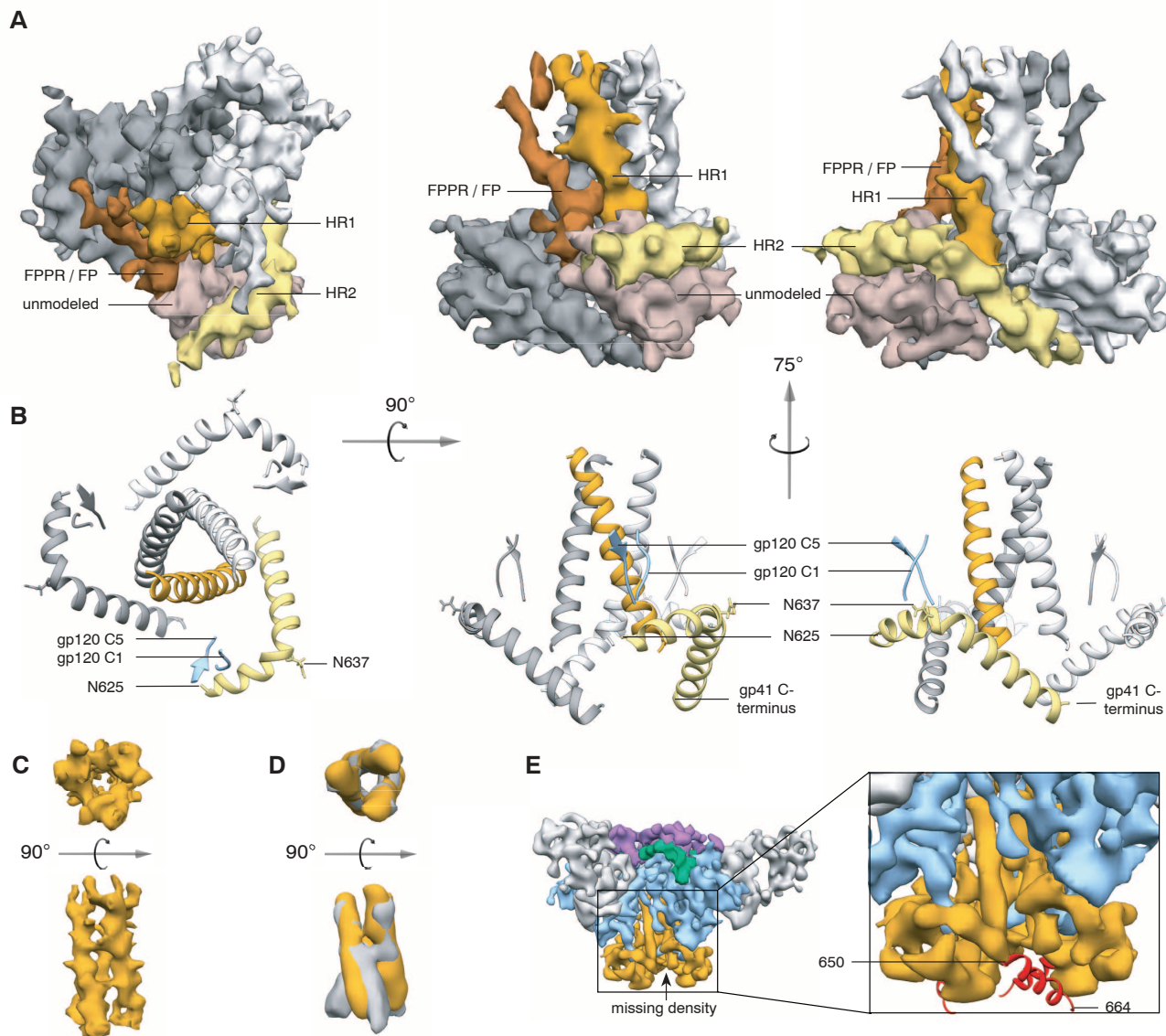


Fig. 3. Structure of gp41. (A) Segmented EM density map of the gp41 trimer with gp120 removed. The C-terminal half of HR1 (rust) forms a three-helix bundle at the center of the trimer; the C-terminal half of HR2 (yellow) forms a helical structure that wraps around the trimer base. Additional density that is not assigned in the model (beige) likely corresponds to the intervening region between HR1 and HR2, including the disulfide loop, as well as C1 and C5 from gp120. Density parallel to HR1 (brown) likely corresponds to the N-terminal half of HR1, the fusion peptide proximal region (FPPR), and the fusion peptide (FP). (B) Modeled portion corresponding to the same

views of the EM density maps in (A). (C) EM density of the three-helix bundle formed by HR1 in the PGV04-bound trimer structure. (D) Overlay of the EM density of the three-helix bundle formed by HR1 in the PGV04-bound structure that is filtered to 9.5 Å (orange) with the 9 Å reconstruction of a 17b-bound SOSIP gp140 trimer (gray, EMDB-5462) (37). (E) An 8.2 Å reconstruction of a SOSIP trimer from which the last 14 amino acids were deleted (SOSIP.650-PGV04). The difference between the SOSIP.650 and SOSIP.664 maps corresponds to a short helical segment (red) at the end of HR2 that projects toward the adjacent protomer (see also fig. S8).

and antibody-binding studies using the corresponding SOSIP.664 gp140 trimers (19).

Immunoglobulin framework insertions and mutations are important for broad and potent neutralization (5). Our structure shows that framework region 3 in the heavy chain (HFR3) of CD4bs antibodies 3BNC117 (also 3BNC60), VRC03, and VRC06 interact with basic residues on an adjacent protomer (Fig. 5, D and E). HFR3 insertions contain acidic residues that point toward conserved basic residues near the V3 tip of the adjacent gp120 protomer (Fig. 5E). A properly folded trimer positions these basic residues in an appropriate quaternary orientation for interacting with CD4bs bnAbs. The preference of VRC06 for cleaved over uncleaved trimers (47) is also consistent with previous observations that cleavage is critical for Env to adopt a native-like conformation (20).

Affinity maturation events in the evolution of the CH103 CD4bs bNAbs involve the early appearance of acidic residues in the HFR3 loop that

then persist (48). However, HFR3 makes no contact with gp120 in the crystal structure of the monomeric gp120-CH103 complex (PDB ID: 4JAN) (48). The trimer structure shows that HFR3 of CH103 is in close proximity to the basic residues on V3 from the adjacent gp120 (Fig. 5E). Thus, the quaternary context of the CH103 epitope helps to explain why somatic hypermutation within HFR3 is critical and why the acidic residues are conserved once they appear.

The V3 interactions and the evolution of framework mutations essential for neutralization breadth and potency are likely to be relevant to many CD4bs bnAbs. The quaternary constraints on these epitopes will affect how potent CD4bs bnAbs, such as VRC01, are induced and then undergo the affinity maturation process.

Conclusions

The 5.8 Å resolution cryo-EM structure presented here is similar to the x-ray structure of the same

SOSIP.664 gp140 trimers in complex with bnAb PGT122 (fig. S2H) (28). Both structures are, however, very different from a 6 Å structure recently reported for a detergent-solubilized, full-length but uncleaved trimer (49). Differences in the design of the Env trimers might contribute to some of these structural inconsistencies. Alternatively, the cryo-EM methodology used to derive the full-length uncleaved trimer structure has been criticized, and the controversy is as yet unresolved (50–54). Considered in isolation, our soluble SOSIP.664 gp140 trimer structures are consistent with known Env structure-function relationships and also provide insight into the prefusion form of the trimer. The structures explain how bnAbs recognize their epitopes and why quaternary constraints prevent some non-neutralizing Abs from binding the trimer. Overall, the structures are a step toward understanding trimeric Env at atomic-level resolution and should guide improvements in Env-based vaccines.

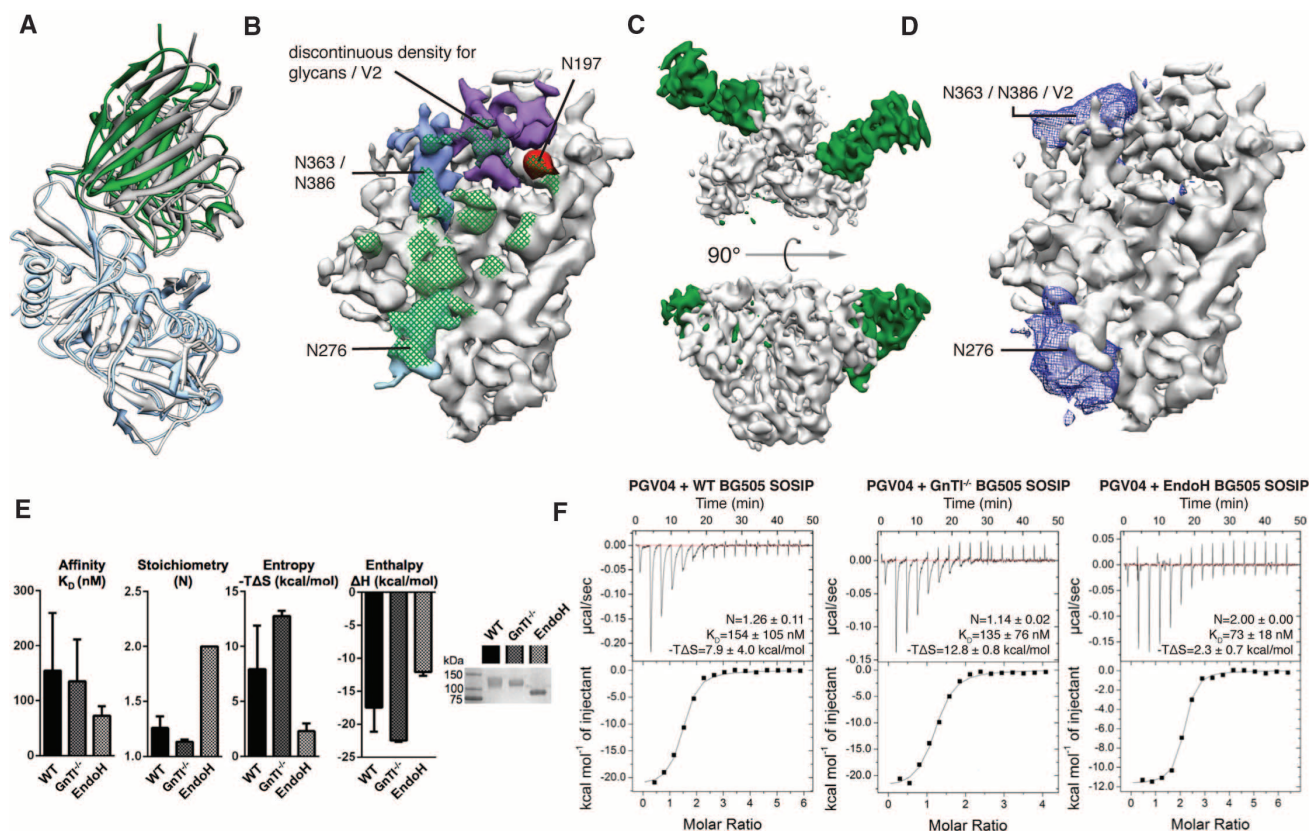


Fig. 4. PGV04 interactions with Env trimer. (A) Superposition of the gp120 portion of the x-ray structure of the gp120:PGV04 complex (3SE9, white/gray) and the EM structure of the SOSIP.664 trimer PGV04 complex (blue/green) illustrates subtle differences in the PGV04:gp120 interaction. Residues 121 to 202 and 395 to 410 are omitted for clarity. (B) Elaborated nature of the CD4 binding site with densities of interest highlighted (red, Asn¹⁹⁷; light blue, Asn²⁷⁶; blue, Asn³⁶³ and Asn³⁸⁶; purple, V2 and/or portions of neighboring protruding glycans). Densities corresponding to glycans and V2 are positioned to influence how the CD4bs is recognized. A glycan at Asn²⁷⁶ makes extensive interactions with the light chain of PGV04. The full PGV04 epitope on the trimer within 5 Å is denoted by green cross-hatching. (C) Top and side views of EM reconstruction of one BG505 SOSIP.664 trimer (white) bound to two PGV04 Fabs (green) at 7.9 Å resolution. (D) Density representing statistically significant differences

between a PGV04-liganded and an unliganded protomer [“t-map” contoured at $P < 0.001$ (31)]. This view is the same as (B). Raw data are shown in fig. S16. Difference density (blue) in the vicinity of Asn²⁷⁶, Asn³⁶³/Asn³⁸⁶, and V2 is apparent when PGV04 is bound at the CD4bs. (E) Effect of glycosylation on PGV04 binding to the SOSIP.664 gp140 trimer, as evaluated by ITC. PGV04 binds a deglycosylated version of the same trimer with slightly higher affinity and stoichiometry, as well as reduced entropy. Bar graphs represent the means ($\pm$ SD) for the binding affinity, stoichiometry, entropy, and enthalpy derived from at least two independent titrations. Non-reducing SDS–polyacrylamide gel electrophoresis (PAGE) shows the difference in molecular weight when the trimer is produced in 293T cells (WT) or in 293S GnTI⁻ cells before and after deglycosylation with EndoH. (F) Raw data (top) and binding isotherms (bottom) for representative ITC binding experiments.

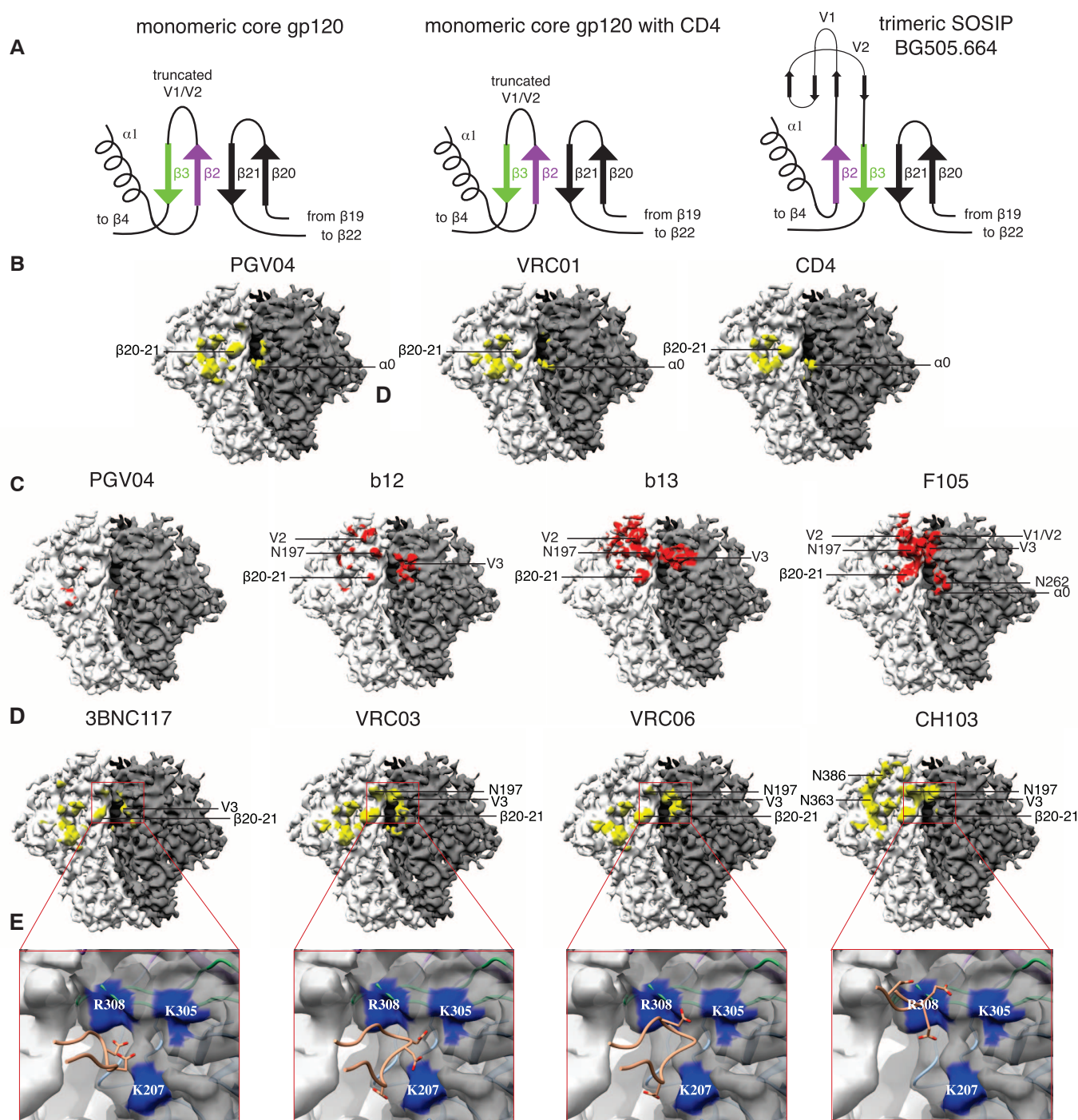


Fig. 5. Quaternary nature of the CD4 binding site. (A) Relative to gp120 monomer structures (e.g., PDB IDs 1GC1 and 3TGT), the bridging sheet has a different topology in the trimer, as illustrated by the cartoon models. Trimer formation alters how the base of V2 ($\beta 2$ – $\beta 3$) and $\beta 20$ – $\beta 21$ are arranged relative to the CD4bs (see also fig. S18). (B) In the EM reconstruction of the trimer, CD4 and CD4bs bnAbs bound to one protomer are in close proximity to the adjacent protomer. The gp120-CD4bs Fab x-ray structures were docked into the EM map via alignment of the gp120 portion of the structures. All regions of the EM map within 5 Å of PGV04, VRC01, and CD4 are colored yellow and are similar to the areas within 5 Å as observed in the corresponding x-ray structures (fig. S20 shows all areas within 2 Å). (C) Major clashes with some CD4bs antibodies differ markedly in the trimeric context. Areas of the EM map within 2 Å of the Fabs are colored red (fig. S20 shows all areas within 5 Å). The minimal clashes with PGV04 serve as a comparator. b12 has only a few clashes

with V1/V2, glycans, and V3 from the adjacent protomer, whereas b13 and F105 have extensive clashes. Note that although b12 is typically a bnAb, it does not neutralize the BG505 virus or bind the BG505 SOSIP.664 trimer (19). Thus, the clashes that we visualize here are consistent with each antibody being unable to neutralize the corresponding virus (19). (D) Areas in the EM map within 5 Å of docked Fabs, all of which contain acidic HFR3 insertions, are colored yellow (fig. S20 shows all areas within 2 Å). In addition to their previously known CD4bs epitopes, these bnAbs also interact with the V3 tip and proximal region near the protomer interface (red box). (E) bnAbs from (D) contain acidic HFR3 residues that interact with the basic residues in the highlighted area. Close-up views show the antibody HFR3 interactions with basic residues in the trimer highlighted in the red boxed regions in (D). Acidic residues in the HFR3 are displayed as sticks; regions in the EM map within 3 Å of basic residues are colored blue and labeled accordingly.

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REPORTS

Detection of Berry's Phase in a Bulk Rashba Semiconductor

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The motion of electrons in a solid has a profound effect on its topological properties and may result in a nonzero Berry's phase, a geometric quantum phase encoded in the system's electronic wave function. Despite its ubiquity, there are few experimental observations of Berry's phase of bulk states. Here, we report detection of a nontrivial π Berry's phase in the bulk Rashba semiconductor BiTeI via analysis of the Shubnikov–de Haas (SdH) effect. The extremely large Rashba splitting in this material enables the separation of SdH oscillations, stemming from the spin-split inner and outer Fermi surfaces. For both Fermi surfaces, we observe a systematic π -phase shift in SdH oscillations, consistent with the theoretically predicted nontrivial π Berry's phase in Rashba systems.

Quantum mechanical systems undergoing adiabatic evolution on a closed path in parameter space acquire a geometrical phase known as Berry's phase, ϕ_B (1, 2). A number of emergent phenomena—including the anomalous

(3) and quantum (4) Hall effects, charge pumping (5), and topological insulating and superconducting phases (6)—are driven by a nontrivial (that is, nonzero) ϕ_B . A nontrivial ϕ_B can, for example, be realized for charge carriers that have k -space cy-

clotron orbits enclosing a Dirac point (7–10). In general, any closed cyclotron orbit is quantized under an external magnetic field B , according to the Lifshitz-Onsager quantization rule

$$A_n \frac{h}{eB} = 2\pi \left(n + \frac{1}{2} - \frac{\phi_B}{2\pi} \right) = 2\pi(n + \gamma) \quad (1)$$

Here A_n is the extremal cross-sectional area of the Fermi surface (FS) related to the Landau level (LL) n ; γ is defined as $\gamma = \frac{1}{2} - \frac{\phi_B}{2\pi}$ and can take values from 0 to 1, depending on the value of

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ϕ_B (7); $\hbar$ is Planck's constant h divided by 2π ; and e is the elementary charge. The quantity γ can be experimentally accessed by analyzing the LL fan diagram of Shubnikov–de Haas (SdH) oscillations. A nontrivial Berry's phase has been observed in pseudo-spin Dirac systems such as graphene (10), as well as elemental bismuth (11), bulk SrMnBi_2 (12), and, potentially, graphite (13–15), although in that case the experimental situation remains unresolved. Clear detection in physical-spin Dirac systems such as topological insulators has been complicated by large Zeeman energy effects and bulk conduction (16–19).

Systems described by the Rashba Hamiltonian also possess a Dirac point and provide an alternative path to realizing a nontrivial ϕ_B . The Dirac point in this class of noncentrosymmetric systems results from the crossing between energy bands spin-split by the Rashba spin-orbit Hamiltonian $H_R = \frac{\lambda}{\hbar} \mathbf{e} \cdot (\mathbf{s} \times \mathbf{p})$, where λ is the Rashba parameter, $\mathbf{e}$ is the unit vector along which the system breaks inversion symmetry (20), $\mathbf{s}$ is the spin, and $\mathbf{p}$ is the momentum vector. Because of this interaction, the energy bands are linearly dispersed around $\mathbf{p} = 0$, and the resulting FS consists of two pockets, an inner FS (IFS) and an outer FS (OFS). For each FS, $\mathbf{s}$ is locked to be normal to $\mathbf{p}$, thereby forming a helical spin texture around the Dirac point at $\mathbf{p} = 0$. It has been predicted that in systems where both Rashba and Dresselhaus spin-orbit interactions are present, both FSs obtain a Berry's phase expressed as $\Phi_B = \frac{\lambda^2 - \beta^2}{|\lambda^2 - \beta^2|} \pi$, with β corresponding to the Dresselhaus parameter (21–23). Accordingly, in the case of pure Rashba spin splitting (RSS) ($\beta = 0$), both inner and outer FSs are expected to obtain a nontrivial π Berry's phase. Consequences of this Rashba coupling and phase have been studied in two dimensions in

semiconductor heterostructures using weak anti-localization (24), ensemble averaging in interferometers (25), commensurability oscillations (26), and SdH oscillations (27, 28). Given the relatively small RSS in these systems, the IFS and OFS have similar areas, and the SdH oscillations show beating patterns that obscure the underlying oscillation index structure. In addition, when the spin is aligned to the external magnetic field by a larger Zeeman energy, RSS disappears, and the Berry's phase takes on the trivial value.

Recently, a large RSS in a bulk material has been found in the polar semiconductor BiTeI (29–32). This material is composed of alternating Bi, Te, and I atoms stacked along the hexagonal c axis and is typically electron-doped by native defects, as found in many chalcogenide semiconductors. Because of the absence of inversion symmetry and the strong polarity of the system, accompanied by the strong spin-orbit interaction of Bi, an extremely large RSS occurs around the hexagonal face center of the Brillouin zone, referred to as the A point (30). Both angle-resolved photoemission spectroscopy and optical spectroscopy (29, 31–34) reveal that the RSS in BiTeI approaches 400 meV, with a Dirac point located ~ 110 meV above the conduction band minimum, in good agreement with relativistic first-principles calculations (30, 35).

Figure 1 shows the calculated band dispersion around the A point (Fig. 1A) and a typical FS for a Fermi level E_F located slightly above the Dirac point (Fig. 1B), corresponding to the samples measured here. The large RSS causes the ratio of the extremal cross-sectional areas of the IFS and OFS to be extremely large, diverging as E_F approaches the Dirac point. In terms of SdH experiments for samples with finite electron mobility, this conve-

niently decouples the two sets of oscillations by confining the IFS and OFS to the low- and high-field regime, respectively. Moreover, the observed giant RSS in BiTeI can dominate the Zeeman effect, even in high magnetic fields. Therefore, BiTeI is an ideal system for investigating the Berry's phase originating from the Rashba spin-split band.

Figure 2 shows the in-plane magnetoresistivity (ρ_{xx}) of a BiTeI sample (sample A) up to 14 T applied along the [001] c axis at 1.8 K. In this sample, the low-field Hall mobility is $\sim 300 \text{ cm}^2/\text{V}\cdot\text{s}$ at 1.8 K. The Hall density ($\sim 4.0 \times 10^{19} \text{ cm}^{-3}$) would place E_F slightly above the Dirac point. As can be seen, the SdH oscillations stemming from the IFS (< 4 T) and OFS (> 10 T) are well separated from each other; thus, they can be separately analyzed. Focusing first on the IFS, Fig. 3A shows the SdH oscillations at various temperatures. Because the IFS extremal cross-sectional area (A_{IFS}) is so small, 3.4 T is sufficient to reach the quantum limit, where all electron states in the IFS are condensed into the lowest LL. This can be seen by taking the negative second derivative of resistivity ($-d^2\rho_{xx}/dB^2$) (Fig. 3B). Here, the lowest-index maximum (corresponding to the peak in resistivity) appears at 3.4 T ($1/B = 0.294 \text{ T}^{-1}$), and the oscillation disappears above 5 T. The period of the oscillation [$\Delta(1/B) = 0.294 \text{ T}^{-1}$] corresponds to $A_{\text{IFS}} = 3.1 \times 10^{-4} \text{ \AA}^{-2}$.

The oscillatory component $\Delta\rho_{xx}$ is plotted in Fig. 3C, after subtraction of the nonoscillating background deduced by fitting a fourth-order polynomial, based on the resistivity values at the node positions of the SdH oscillations (Fig. 3A). From the temperature dependence of the oscillation amplitude at 3.4 T (Fig. 3D), the electron effective mass m^* for the IFS (m_{IFS}^*) is determined to be $(0.023 \pm 0.001)m_0$ (where m_0 is the free electron mass), following the Lifshitz-Kosevich formula for a three-dimensional (3D) system (36–38)

$$A(B, T) = \frac{\Delta\rho}{\rho_0} \propto (\hbar\omega_c/E_F)^{\frac{1}{2}} \exp(-2\pi^2 k_B T_D / \hbar\omega_c) \frac{2\pi^2 k_B T / \hbar\omega_c}{\sinh(2\pi^2 k_B T / \hbar\omega_c)} \quad (2)$$

Here, ρ_0 is the nonoscillatory component of the resistivity at $B = 0$, T_D is the Dingle temperature, k_B Boltzmann's constant, and the cyclotron frequency $\omega_c = eB/m^*$. Considering $A_{\text{IFS}} = 3.1 \times 10^{-4} \text{ \AA}^{-2}$, first-principles calculations indicate $E_F = 151$ meV above the conduction band minimum. At this E_F , the calculated m_{IFS}^* is $0.021m_0$, in agreement with the observed value. We can thus confidently assign this set of SdH oscillations to the IFS.

In the expression for ρ_{xx} in 3D systems (36–40)

$$\rho_{xx} = \rho_0 [1 + A(B, T) \cos(2\pi(B_F/B - \delta + \gamma))] \quad (3)$$

$1/B_F$ is the SdH frequency, and δ is a phase shift determined by the dimensionality, taking the value $\delta = 0$ (or $\delta = \pm 1/8$) for the 2D (or 3D) case (41–43). In this formula, values of $|\gamma - \delta| = |1/2 - \phi_B/2\pi - \delta|$

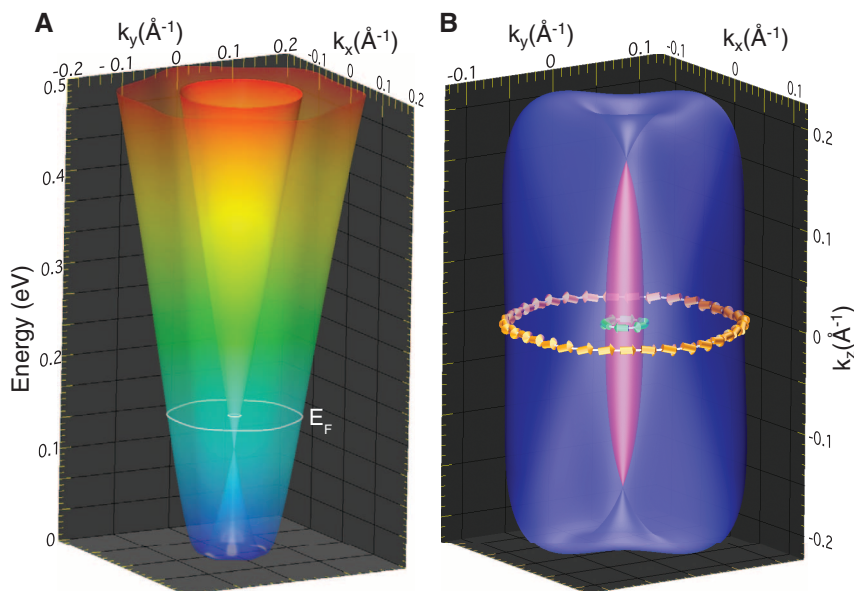


Fig. 1. Electronic structure of BiTeI. (A) Energy band dispersion of the conduction band around the hexagonal face center A point of the BiTeI Brillouin zone. (B) Typical Fermi surface of BiTeI for E_F located above the Dirac point. The Fermi surface consists of an inner Fermi surface (pink pocket) and an outer Fermi surface (purple-blue pocket). The extremal orbits normal to k_z have helical spin textures (arrows), with opposite helicities.

between 0 and $1/8$ indicate a nontrivial π Berry's phase, with the precise value determined by the degree of two-dimensionality via δ . To obtain the Berry's phase, the relation between $1/B$ and Landau index number n is plotted in Fig. 3E. Here, peak and valley positions of the oscillation are determined using $-d^2\rho_{xx}/dB^2$ (Fig. 3B). We assign integer indices to the ρ_{xx} peak positions in $1/B$ and half integer indices to the ρ_{xx} valley positions. The interpolation line of n versus $1/B$ in sample A has an intercept between $-1/8$ and 0, indicating a nontrivial Berry's phase for the IFS. A similar trend is reproduced for other samples B, C, and D with $E_F \sim 244, 194$, and 154 meV, respectively (Fig. 3E). In a pure Rashba system ($\gamma = 0$), these results indicate that δ deviates from the 3D limit $|\delta| = 1/8$,

likely because of the quasi-2D nature of the system. In sample B, the SdH oscillation originating from the IFS can be seen up to 39 T with the respective $\Delta(1/B) = 0.0259 \text{ T}^{-1}$, corresponding to a much larger $A_{\text{IFS}} = 3.65 \times 10^{-3} \text{ \AA}^{-2}$. The strict linearity of this index plot up to the quantum limit is a consequence of negligible Zeeman splitting and the fact that the OFS dominates the variation of the chemical potential at this magnetic field. This allows us to avoid index shifting near the quantum limit, as observed, for example, in graphite (14), in much the same manner as has been observed for specific field orientations of bismuth (11).

Next, we turn to SdH oscillations originating from the OFS. These oscillations are clearly observed in the higher-magnetic field

region. Figure 4A shows ρ_{xx} of sample A up to 56 T at various temperatures. In this case, clear SdH oscillations are observed above 10 T at 1.5 K and can be discerned even at 100 K above 40 T. The oscillatory component is deduced by subtracting a fourth-order polynomial, as discussed previously, and is plotted as a function of $1/B$ in Fig. 4B. The period of the oscillation [$\Delta(1/B) = 0.00288 \text{ T}^{-1}$] corresponds to an OFS extremal cross-sectional area of $A_{\text{OFS}} = 3.4 \times 10^{-2} \text{ \AA}^{-2}$, which is very consistent with the calculated $A_{\text{OFS}} = 3.43 \times 10^{-2} \text{ \AA}^{-2}$ at $E_F = 151$ meV. This provides an important self-consistency check, given that this E_F was derived from the IFS SdH results. From the temperature dependence of the peak amplitude at 53.6 T, m_{OFS}^* is determined to be $(0.183 \pm 0.003)m_0$ (Fig. 4C), also in good agreement with the calculated value $m^* = 0.182m_0$. To obtain the Berry's phase for OFS, the fan diagram is plotted in Fig. 4D. Because a longer extrapolation is required in the case of the OFS, we measured five samples with varying E_F for the determination of the intercept value. Here the integer index n , corresponding to the ρ_{xx} maximum, is assigned such that a linear extrapolation of the index plots yields an intercept closest to zero index ($n = 0$). As can be seen in Fig. 4D, the index plots uniformly exhibit a linear dependence on n with the lowest integer index $n = 6$. This linearity again suggests that the Zeeman effect is negligible and that the observed oscillations are far from the OFS

Fig. 2. SdH oscillations for the inner and outer Fermi surfaces in BiTeI.

Transverse magnetoresistivity (ρ_{xx}) of BiTeI with $B \parallel [001]$ at 1.8 K. Two types of SdH oscillations (indicated by arrows) originate from the inner and outer Fermi surfaces, as illustrated in Fig. 1B.

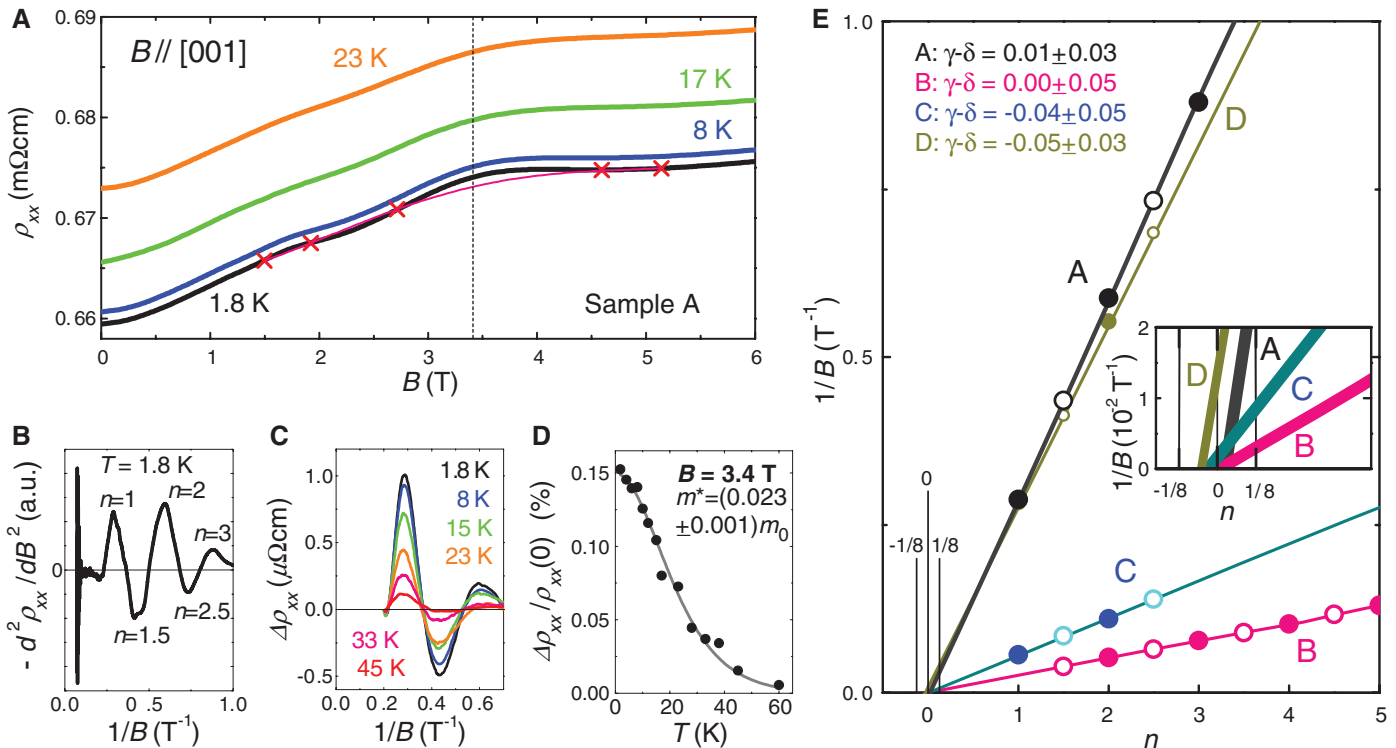
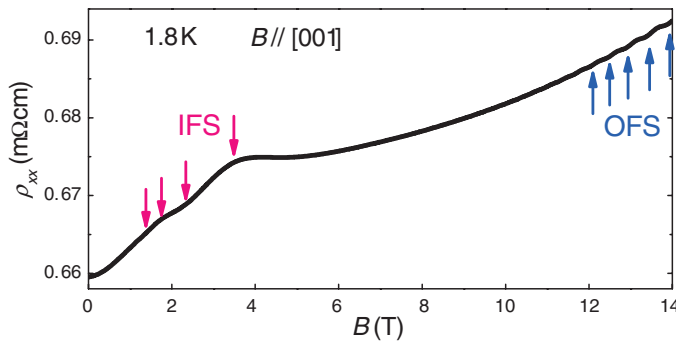


Fig. 3. Berry's phase of the inner Fermi surface. (A) ρ_{xx} of sample A with $B \parallel [001]$ in the lower-magnetic field region, at various temperatures. The nonoscillatory background component is deduced at each temperature based on the SdH node positions (crosses), as shown in the case at 1.8 K. (B) Negative second derivative of ρ_{xx} ($-d^2\rho_{xx}/dB^2$) as a function of $1/B$. a.u., arbitrary units. (C) Oscillatory component at various temperatures. (D) Tem-

perature dependence of the SdH oscillation amplitude at 3.4 T [vertical dashed line in (A)]. (E) Landau index plot of the IFS (error bars are smaller than the symbol size). Closed circles denote the integer index (ρ_{xx} peak), and open circles indicate the half integer index (ρ_{xx} valley). The intercept is between $-1/8$ and $1/8$ for samples A and D, as well as for samples with a higher E_F (samples B and C). (Inset) Magnified view around the intercept.

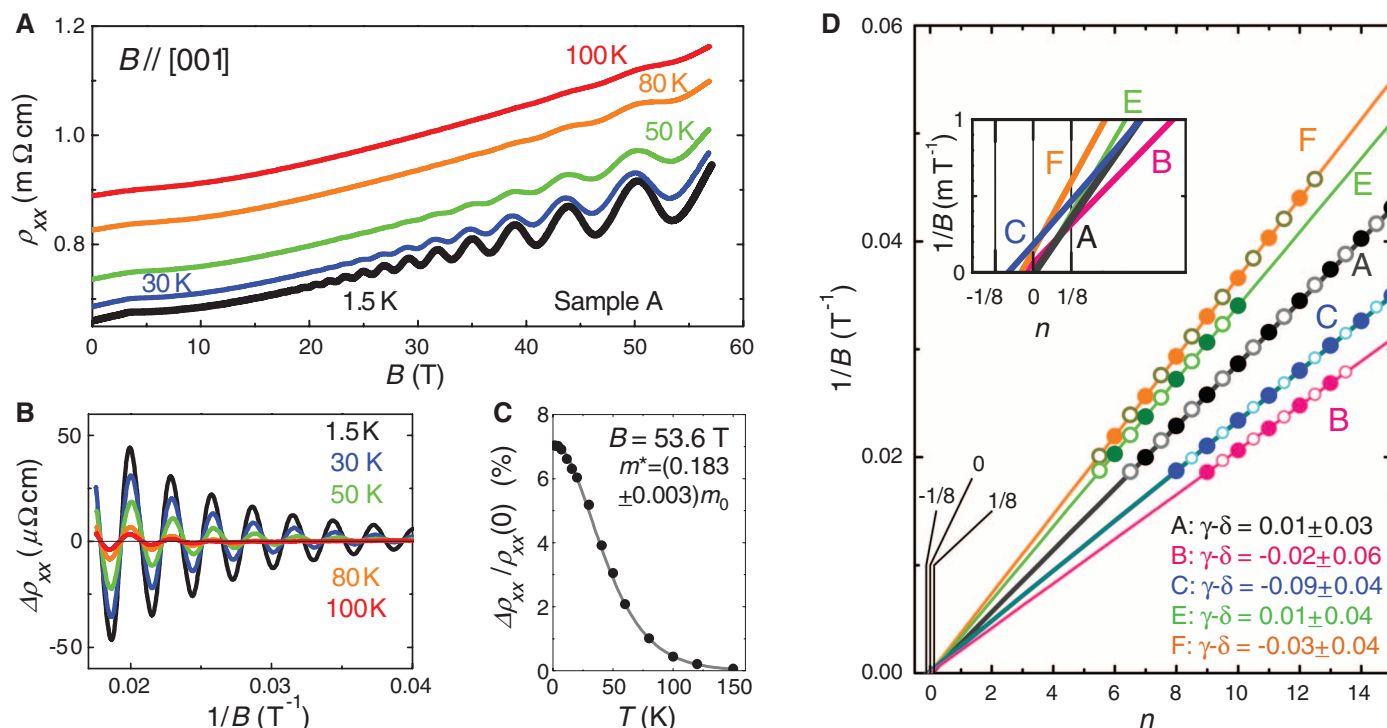


Fig. 4. Berry's phase of the outer Fermi surface. (A) ρ_{xx} of sample A with $B \parallel [001]$ up to 56 T at various temperatures. (B) The oscillatory component as a function of $1/B$. (C) Temperature dependence of the oscillation amplitude at 53.6 T. (D) Landau index plots of the OFS with various E_F samples A, B, C, E, and F. Closed circles denote the integer index (ρ_{xx}

peak), and open circles indicate the half integer index (ρ_{xx} valley). The integer index n is assigned such that a linear extrapolation of the index plots yields an intercept closest to zero index ($n = 0$). (Inset) Magnified view around the intercept, showing an intercept between $-1/8$ and $1/8$ for all samples.

quantum limit. Similar to the case of the IFS, here again the intercept $|\gamma - \delta|$ is in the range 0 to $1/8$. Thus, we observe a systematic π -phase shift in SdH oscillations, consistent with the theoretical prediction that a pure Rashba system will exhibit a non-trivial π Berry's phase for both the IFS and OFS.

Observation of this effect is made possible by the extremely large Rashba energy in BiTeI. It is interesting to note that, unlike the other candidate spin Berry's phase systems such as semiconductor heterostructures and surface state of topological insulators, BiTeI is a 3D electronic system (albeit, with a quasi-2D electronic structure). Accordingly, this system may provide the opportunity to explore the dependence of Berry's phase on the trajectory-dependent spin evolution. Moreover, the prediction that pressure can drive BiTeI through a quantum phase transition to a topological phase provides a test to compare Berry's phase stemming from different physical origins (35).

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Photon-Mediated Interactions Between Distant Artificial Atoms

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Photon-mediated interactions between atoms are of fundamental importance in quantum optics, quantum simulations, and quantum information processing. The exchange of real and virtual photons between atoms gives rise to nontrivial interactions, the strength of which decreases rapidly with distance in three dimensions. Here, we use two superconducting qubits in an open one-dimensional transmission line to study much stronger photon-mediated interactions. Making use of the possibility to tune these qubits by more than a quarter of their transition frequency, we observe both coherent exchange interactions at an effective separation of $3\lambda/4$ and the creation of super- and subradiant states at a separation of one photon wavelength λ . In this system, collective atom-photon interactions and applications in quantum communication may be explored.

In free space, the interaction of individual atoms with vacuum fluctuations of the electromagnetic field leads to both the relaxation of atomic excited states and the renormalization of atomic energy levels. On one hand, the emission of real photons into a single mode of the electromagnetic continuum at the atomic transition frequency results in spontaneous emission. On the other hand, the emission and absorption of virtual photons from all modes of the continuum gives rise to a Lamb shift (a shift in the energy-level separation). In the presence of additional atoms, both real and virtual photons emitted by one atom can be absorbed by another one, giving rise to nontrivial atom-atom interactions. Such interactions are challenging to observe in three dimensions because of the weak electromagnetic fields generated by individual photons and the poor spatial mode matching between the modes of the emitting and absorbing atoms. Nevertheless, signatures of these interactions in the form of super- and subradiant states, which depended on the separation of two trapped ions, were observed (1, 2). Due to the inverse scaling of the interaction strength with interatomic separation, the super- and subradiant lifetimes were observed to differ by only a few percent in these experiments.

Confining both the radiation field and the two-level systems to one dimension overcomes the aforementioned challenges and allows for observing photon-mediated interactions between atoms. This nascent field of physics known as waveguide quantum electrodynamics (QED) is expected to contribute to the development of quantum networks (3), circuits operating on the level of single photons (4, 5), implementations of quantum memories using electromagnetically

induced transparency (6), and the generation of photon-photon interactions (7).

At optical frequencies, realizations of waveguide QED systems have been proposed for quantum dots interacting with surface plasmons (8–12) and for atoms trapped in the near field of a nanofiber (13–16). Superconducting circuits are also a natural choice to investigate the strong interaction of quantum two-level systems with microwave photons in open one-dimensional (1D) transmission lines (17). Near-unit reflectance of weak coherent fields by a single qubit was first observed by Astafiev *et al.* (18). Subsequently, phenomena such as resonance fluorescence, Autler-Townes splitting, electromagnetically induced transparency (19), time-resolved emission dynamics (20), and

the cross-Kerr effect (21) have been explored, and single-photon routers (22) have been realized.

Here, we demonstrate the coupling between two superconducting qubits mediated by microwave photons in a 1D transmission line. In contrast to the 3D case in which the interaction decreases rapidly with increasing separation between the qubits, the interaction in 1D shows behavior at an approximately constant amplitude [see (23) and references therein]. Indeed, the photon-mediated interaction leads to correlated decay of a pair of qubits at a rate $\gamma \propto \cos(2\pi d/\lambda)$ and coherent exchange-type interactions at a frequency $J \propto \sin(2\pi d/\lambda)$. Whereas the interqubit separation d is fixed for a given sample in our experiment, we vary the effective separation between the qubits in terms of their emission wavelength λ . For this purpose, we change the qubit transition frequencies by an appreciable fraction of their maximal values (Fig. 1A), an aspect that is challenging to achieve in most atomic systems.

We have fabricated a sample in which two superconducting transmon qubits (24) are coupled to a 1D coplanar waveguide transmission line at an interqubit separation of $d = 18.6$ nm (Fig. 1A). For both qubits, we have determined the maximum frequency 6.89 and 6.84 GHz of the ground $|g\rangle$ to first excited state $|e\rangle$ transitions and the identical anharmonicity -298 MHz of the first-to-second excited state transition using spectroscopic techniques.

To further characterize the system, we measured the amplitude and phase of a weak drive field coherently scattered from the sample in a detection bandwidth much less than 1 MHz (25).

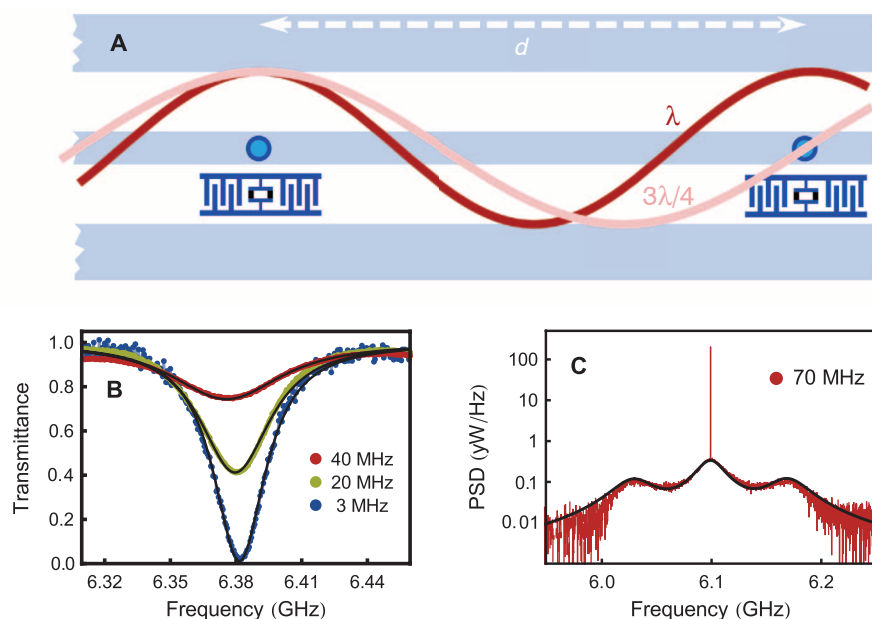


Fig. 1. The experimental system. (A) Schematic of two transmon qubits coupled to an open transmission line at a fixed separation d corresponding to an effective separation of λ or $3\lambda/4$, which is tunable by adjusting the qubit transition frequency. (B) Transmittance spectrum $|t|^2$ of a single qubit measured at the indicated drive rates. (C) Power spectral density (PSD) of the radiation reflected by a single qubit. Red lines are data; black lines denote theory (see text for details). $1 \text{ yW} = 10^{-24} \text{ W}$.

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We determined the transmittance $|t|^2$ and reflectance $|r|^2$ (normalized transmitted and reflected power) from the measured transmission and reflection coefficients t and r (25). When either qubit is detuned by many linewidths from the other, we observed no difference in line shape compared to a single qubit. At 6.4 GHz, the minimum transmittance is less than 0.025 for low drive powers (Fig. 1B), indicating strong coupling (25).

From the linewidth, the decay rates of both qubits are determined to be $\gamma_1/2\pi \approx 26 \pm 1$ MHz at 6.4 GHz, and 13 ± 1 MHz at 4.8 GHz, consistent with expectations for an ohmic environment (18). When the power of the incident drive field is increased, the qubit transition saturates (26), and transmittance increases to unity for large drive powers (Fig. 1B).

When the two qubits are tuned into resonance, such that a photon emitted by one of the qubits may be absorbed by the other, we expect correlated effects to become apparent. For both qubits tuned to either 6.4 or 4.8 GHz, corresponding to an effective qubit separation d of λ or $3\lambda/4$, respectively, we tune the qubits through resonance and plot $|t|^2$ and $|r|^2$ versus frequency (Fig. 2). When the qubits are detuned from each other by much more than the range displayed in Fig. 2, they each display a pronounced well-resolved maximum in $|r|^2$, similar to data obtained for a single qubit. When the two qubits are in resonance with

each other for $d \sim \lambda$, only a single resonance is observed, with $|r|^2$ reaching unity at mutual resonance and $|t|^2$ going to zero (Fig. 2B).

These observations can be understood by considering photon-mediated interactions between the two qubits (23). When $d \sim \lambda$, the exchange interaction $J = 0$ and the correlated decay rate takes its maximal value $\gamma = \gamma_1$. This type of decay leads to subradiant, $|D\rangle = (|ge\rangle - |eg\rangle)/\sqrt{2}$, and superradiant, $|B\rangle = (|ge\rangle + |eg\rangle)/\sqrt{2}$, states (1). When the qubits are separated by λ , they are driven with the same phase by a single drive tone. Therefore, the symmetric state $|B\rangle$, which has the same symmetry as the drive at the qubits, can be excited from the ground state $|gg\rangle$. Thus, this state is bright. In contrast, the matrix element for the antisymmetric state $|D\rangle$, which has opposite symmetry with respect to both the drive field and the resonant vacuum fluctuations that cause relaxation, is zero. Because the latter is dark, the two-qubit system essentially behaves as a single two-level system with ground state $|gg\rangle$ and excited state $|B\rangle$ at low drive powers with the superradiant decay rate $\Gamma_B = 2\gamma_1$ (23). The two-qubit results (Fig. 2B) are qualitatively similar to those of a single qubit (Fig. 1B), but with a linewidth $\Gamma_B/2\pi \sim 52 \pm 1$ MHz, which is twice as large as the one extracted for a single qubit. This is a clear signature of superradiance.

The situation is more subtle when the qubits are separated by $d \sim 3\lambda/4$ or any odd multiple of

$\lambda/4$. Then one of the qubits is at a node of the driving field, whereas the other is at an antinode (Fig. 1A). In this case, the above argument leading to the creation of bright and dark states does not apply, and correlated decay is absent with $\gamma = 0$. Instead, the coherent exchange interaction between qubits mediated by virtual photons takes its maximal value $J = \gamma_1/2$ (27).

The expected signature of this coherent exchange interaction is an anticrossing of the qubit energy levels similar to the one observed for two qubits coupled to a resonator in a circuit QED system (28, 29). As the expected splitting $2J = \gamma_1$ can be only as large as the peak width γ_1 , this signature is not apparent in the elastic scattering data. The doublet observed in reflection in Fig. 2D is a consequence of dressing of the two-qubit system by the input field rather than a signature of exchange interaction (25). The quantitative analysis presented in (23) (solid black lines) agrees with the data (colored lines) over the full range of frequencies shown in Fig. 2.

To further characterize photon-mediated atom-atom interactions, the full spectrum of the elastically and inelastically scattered radiation, including the Rayleigh scattered and resonance fluorescence contributions, has been recorded in both reflection and transmission (25). In a reference measurement of the resonance fluorescence spectrum of a single qubit, we observe the standard Mollow triplet (30), including a δ -like peak due to elastically (Rayleigh) scattered radiation (Fig. 1C). The Mollow sidebands appear at a detuning

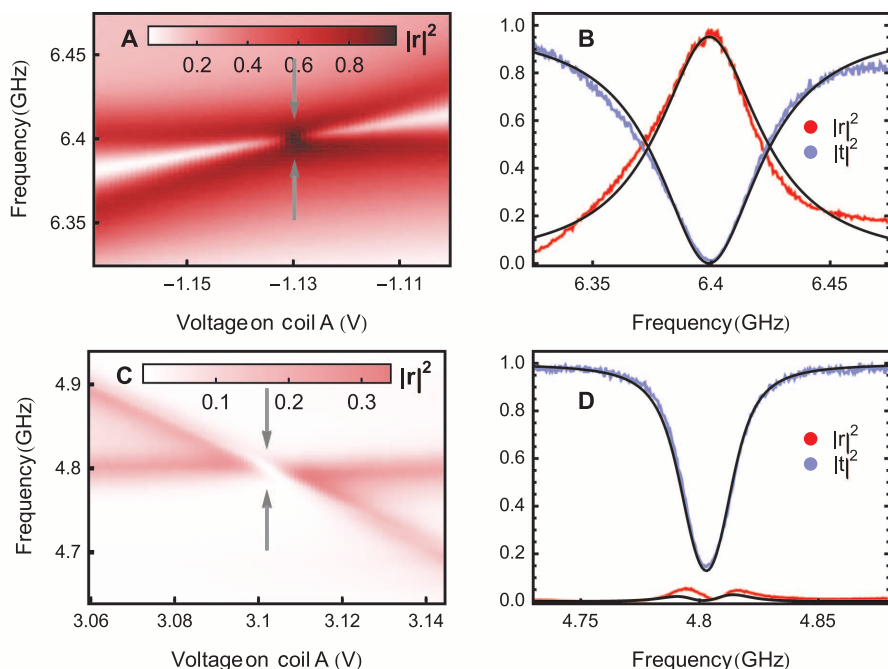


Fig. 2. Elastically scattered radiation. Reflectance spectra $|r|^2$ of the two-qubit system [(A) and (B)] at $d \sim \lambda$ recorded with a drive rate of 7.5 MHz and [(C) and (D)] at $d \sim 3\lambda/4$ with rate 8.7 MHz (red data sets). In (B) and (D), the transmittance $|t|^2$ is also shown (blue data sets). In (A) and (C), the frequency of one qubit is tuned by applying the indicated voltages to millimeter-size coils integrated in the sample mount, whereas the other qubit is kept at a fixed frequency. Close to resonance, interference effects cause the qubit peaks to become asymmetric. (B) and (D) show spectra at bias points indicated by arrows in (A) and (C), respectively. Colored lines are data; black lines denote theory (see text for details).

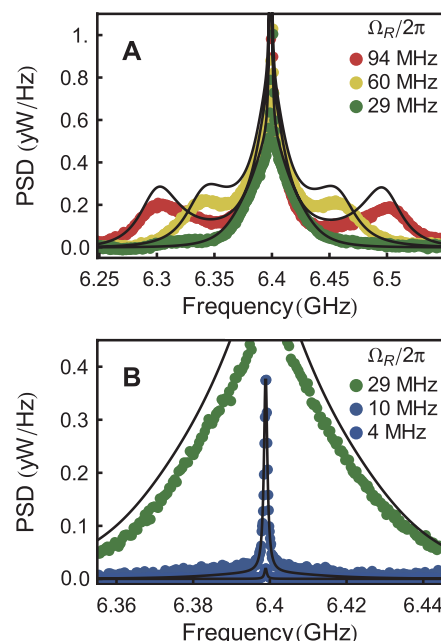


Fig. 3. Super- and subradiance. (A and B) Power spectral density measured in reflection for two qubits in resonance at $d \sim \lambda$ at the indicated Rabi drive rates Ω_R . Solid lines are numerical calculations (23) using the parameters specified in the supplementary materials (25).

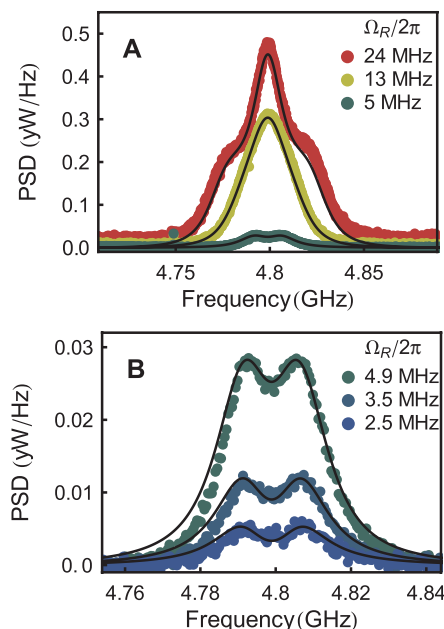


Fig. 4. Exchange interaction. (A and B) Power spectral density measured in transmission for two qubits in resonance at $d \sim 3\lambda/4$ at the indicated Rabi drive rates Ω_R . Solid lines are as in Fig. 3.

from the center peak corresponding to the Rabi frequency induced by the drive.

When both qubits are tuned to 6.4 GHz corresponding to $d \sim \lambda$, we observe a Mollow triplet-like spectrum with a narrow resonance superimposed at its center frequency (Fig. 3). The narrow resonance becomes more discernible as the drive power is decreased (Fig. 3B). Here and below, the Rayleigh scattered contribution was removed from the data for clarity. These two distinct features, the Mollow triplet and narrow resonance, are due to the formation of super- and subradiant states, respectively.

The effective two-level system $\{|gg\rangle, |B\rangle\}$ is strongly dressed by the drive field, resulting in a Mollow triplet. The width ~ 52 MHz of the main peak, obtained by fitting to numerical calculations (black lines in Fig. 3), is consistent with the value of Γ_B extracted above. Ideally, the dark state $|D\rangle$ is neither excited by the drive nor does it decay into $|gg\rangle$ due to selection rules. In practice, however, it is weakly populated due to qubit dephasing, nonradiative decay from the state $|ee\rangle$, and unequal single-qubit relaxation rates (23). As a result, the dark state $|D\rangle$ appears as a narrow resonance superimposed on the bright-state Mollow triplet. Its linewidth, when compared to numerical results, is approximately $\Gamma_D/2\pi \sim 0.4 \pm 0.2$ MHz. We obtain adequate quantitative agreement between the measured spectra and theory (see lines in Fig. 3) and find the ratio between super- and subradiant lifetimes to be as high as $\Gamma_B/\Gamma_D \geq 100$.

When both qubits are tuned to 4.8 GHz, corresponding to $d = 3\lambda/4$, the resonance fluorescence spectra at high powers ($\Omega_R/2\pi \geq 15$ MHz, where Ω_R is the Rabi drive rate) also display the

expected Mollow triplet features (Fig. 4A). At drive powers much lower than the relaxation rate $\Omega_R/2\pi \leq 5$ MHz, the fluorescence spectrum displays a double-peak structure split by ~ 15 MHz (see Fig. 4B). This observation is a clear signature of the effective exchange interaction J between the two qubits mediated by virtual photons. The observed splitting is slightly larger than the expected value $2J/2\pi = \gamma_1/2\pi \approx 13$ MHz, which is consistent with theory predicting the splitting to be larger than $2J$ in transmission and smaller in reflection (23).

Our results present compelling evidence of strong interaction effects between two superconducting qubits separated by as much as a full wavelength $\lambda \sim 18.6$ mm in an open 1D environment. The observation of these effects presents opportunities to explore decoherence-free subspaces and entanglement over long distances in open environments and could lead to exciting applications in quantum communication technology.

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Supplementary Materials

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Relaxation Mechanism of the Hydrated Electron

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The relaxation dynamics of the photoexcited hydrated electron have been subject to conflicting interpretations. Here, we report time-resolved photoelectron spectra of hydrated electrons in a liquid microjet with the aim of clarifying ambiguities from previous experiments. A sequence of three ultrashort laser pulses (~ 100 femtosecond duration) successively created hydrated electrons by charge-transfer-to-solvent excitation of dissolved anions, electronically excited these electrons via the $s \rightarrow p$ transition, and then ejected them into vacuum. Two distinct transient signals were observed. One was assigned to the initially excited p -state with a lifetime of ~ 75 femtoseconds, and the other, with a lifetime of ~ 400 femtoseconds, was attributed to s -state electrons just after internal conversion in a nonequilibrated solvent environment. These assignments support the nonadiabatic relaxation model.

The hydrated electron e_{aq}^- is a species of fundamental interest in the chemistry of water. It has been implicated in phenomena ranging from aerosol nucleation to radiation

damage in DNA (*1*). As the simplest quantum solute, with only a single electronic degree of freedom, it has been the focus of many experimental and theoretical studies over the years (*2–4*). None-

theless, many of its key attributes remain controversial. For example, the standard picture (5) of an electron residing in a cavity of radius ~ 2.4 Å has been repeatedly questioned (6). Another unresolved issue concerns the relaxation mechanism of e_{aq}^- subsequent to electronic excitation. This mechanism, which represents a subtle interplay between solute-solvent interactions and electronically non-adiabatic dynamics, is of critical importance in hydrated electron chemistry and radiation biology, given that excited states of e_{aq}^- are considerably more reactive than its ground state (7). The relaxation dynamics of e_{aq}^- upon excitation have been studied in bulk water by using transient absorption (TA) (8–10) and resonance Raman spectroscopy (11). Complementary studies have also been carried out in size-selected water cluster anions by using time-resolved photoelectron spectroscopy (TRPES) (12). In this work, we connect these very disparate experimental techniques using TRPES of hydrated electrons in liquid water microjets in order to resolve key questions regarding the relaxation mechanism of e_{aq}^- .

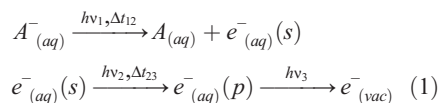
The hydrated electron has a characteristic electronic spectrum peaking at 720 nm that is attributed to excitation from its ground s -state to a manifold of excited p -states within the solvent cavity (13). The proposed relaxation mechanism after $s \rightarrow p$ excitation is shown in Fig. 1. It comprises solvent relaxation in the p -state, $p \rightarrow s$ internal conversion (IC), and solvent relaxation in the s -state. These three processes are characterized by time constants τ_p , τ_{IC} , and τ_s . In TA experiments by Barbara and coworkers (8), the $s \rightarrow p$ transition was excited with a femtosecond pump pulse, and the resulting dynamics were followed by the absorption of a broadband femtosecond probe pulse. Three time scales of 50 to 80 fs, 200 to 400 fs, and ~ 1.1 ps were identified and have been largely reproduced by other laboratories (9, 10). However, the assignment of these lifetimes to particular physical phenomena has been a matter of some debate. Two basic models (Fig. 1) are proposed: the “adiabatic” model and the “nonadiabatic” model (2). The adiabatic model assigns the rapid time scale (50 to 80 fs) to τ_p , the intermediate time scale to τ_{IC} , and the slow time scale to τ_s . Alternatively, the nonadiabatic model assigns the fastest observable time scale to τ_{IC} and the slower two to τ_s . From TA alone, one cannot easily distinguish between the two models, and differing theoretical treatments have favored both models (14, 15).

TRPES of anionic water clusters ($H_2O)_n^-$, provides a different perspective on hydrated electron relaxation dynamics (16–19). In these experiments, the excess electron is electronically excited with a femtosecond pump pulse and then photodetached with a femtosecond probe

pulse. The resulting time-dependent photoelectron spectra show a transient feature clearly associated with the cluster excited state and directly yield τ_{IC} at each cluster size. These experiments show that τ_{IC} decreases from 190 to 60 fs with increasing cluster size up to 200 water molecules. Extrapolating this trend to the bulk ($n \rightarrow \infty$) limit implies $\tau_{IC} \sim 60$ fs for e_{aq}^- . This trend suggests that the fastest time constant seen in the TA experiments corresponds to τ_{IC} , which is consistent with the nonadiabatic relaxation model (Fig. 1).

The validity of this conclusion depends on whether water cluster anions are in fact gas-phase analogs of e_{aq}^- , which is a subject of considerable discussion (2, 20, 21). Recent experiments on liquid water microjets (22) have tested this correspondence directly by using photoelectron spectroscopy to measure the vertical detachment energy (VDE) of ground state hydrated electrons in liquid jets (23–26). The value obtained, 3.3 to 3.5 eV, agrees well with the extrapolated VDE obtained from photoelectron spectra of water cluster anions (18, 27, 28) and raises the question of whether the cluster dynamics will also extrapolate to an observable bulk quantity. We report TRPES experiments on liquid water jets that directly yield the p -state lifetime of e_{aq}^- , resolving which of the two models in Fig. 1 is more appropriate. Our results provide the missing link between the time-resolved water cluster anion experiments and the TA work on bulk hydrated electrons.

The principle of the experiment, in which three femtosecond laser pulses interact with a liquid water jet, is outlined in Fig. 2 and in Eq. 1:



The first pulse, $h\nu_1$, is centered at 239 nm and generates hydrated electrons via charge transfer to solvent (CTTS) excitation of a precursor anion; results are reported here for a 100 mM solution of $Fe(CN)_6^{4-}$. The time (Δt_{12}) between $h\nu_1$ and the pump pulse $h\nu_2$ (800 nm, 1.55 eV, and 85 fs) is held at 200 ps so as to ensure a population of equilibrated, ground-state hydrated electrons (29). The pump pulse lies within the $s \rightarrow p$ absorption

band and excites an electron to the p -state manifold. The probe pulse $h\nu_3$ (266 nm, 4.65 eV, and 125 fs) then detaches the electron to vacuum. The resulting photoelectron kinetic energy (eKE) distribution is measured as a function of Δt_{23} . Because TRPES measures the energy of populated states relative to vacuum, electrons ejected from the p -state will have higher eKE than those ejected from the s -state, which enables us to distinguish between them. The experimental set-up comprises a liquid jet source described elsewhere (25, 30), a 1-kHz femtosecond laser system, and a magnetic bottle electron spectrometer (26, 31).

Shown in Fig. 3 are TRPE spectra using two background subtraction schemes. The eKE scale is corrected for the liquid jet streaming potential as described previously (30, 32). The photoelectron spectrum is shown in Fig. 3A, with the individual one-color contributions from $h\nu_1$ and $h\nu_3$ subtracted from the three-pulse spectra. Each ultraviolet beam causes a delay-invariant two-photon signal from detachment of the precursor anion, which we treat as background. A short-lived transient feature above 2.0 eV and a broad, intense feature centered at 1.2 eV are shown in Fig. 3A. The latter corresponds to a VDE ($=h\nu_3 - 1.2$ eV) of 3.45 eV and is readily assigned to detachment from the s -state of e_{aq}^- on the basis of previous work (23–26). Recent experiments (33, 34) suggest that the escape depth in liquid water for photoelectrons in this eKE range (1 to 3 eV) is at least 5 nm, which is approximately a factor of 20 larger than the cavity radius of e_{aq}^- . These results imply that the detached electrons seen here are not predominantly sampled from the jet surface.

The result of subtracting the ($h\nu_1 + h\nu_3$) two-pulse spectrum from the three-pulse spectrum is shown in Fig. 3B at each delay time. These difference spectra show how the photoelectron signal changes with the addition of the pump pulse, $h\nu_2$. Here, positive signal is induced by $h\nu_2$, whereas negative-going signal represents depletion by $h\nu_2$. The depleted signal overlaps the ground-state feature in Fig. 3A. The positive signal in Fig. 3B exhibits a shoulder from 2.3 to 2.6 eV at the earliest delays that disappears within 100 fs. By 230 fs (Fig. 3B, green trace), the signal has evolved into a smaller transient feature peaking

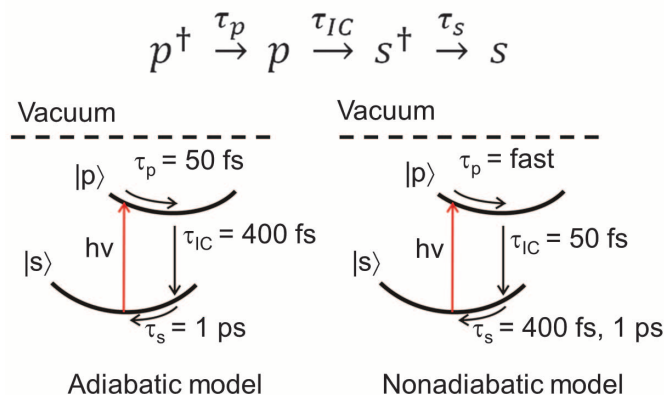


Fig. 1. Proposed relaxation mechanism of the electronically excited hydrated electron. Initial p -state solvent relaxation is followed by IC and then s -state solvent relaxation. Adiabatic (left) and nonadiabatic (right) models differ primarily in τ_{IC} .

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around 1.7 eV that continues to decay and shift toward lower eKE on a significantly longer time scale. The early-time behavior of the shoulder at high eKE can be more readily discerned by subtracting the curve at 230 fs from those at earlier times. The result, shown in Fig. 3C, is a peak centered around 2.5 eV.

To gain further insight into these dynamics, integrated signal is shown in Fig. 4 as a function of delay over three energy intervals indicated in Fig. 3A. Data are shown as points, and the solid lines are fitting functions. These regions are well fit by a simple, sequential three-step mechanism ($I \xrightarrow{\tau_1} II \xrightarrow{\tau_2} III$), with time constants $\tau_1 = 75 \pm 20$ fs and $\tau_2 = 410 \pm 40$ fs. In Fig. 4, the blue curve is associated with I in the mechanism, red with II, and green with III. The fitting functions for each step in the mechanism are a convolution of the measured pump-probe cross-correlation (115 fs), with the kinetic rate equation for each step (supplementary text).

The blue curve in Fig. 4 corresponds to eKEs separated from the ground-state feature by $\sim h\nu_2$ and is assigned to the initially excited p -state. The green curve indicates that the ground state is

initially depleted by the pump pulse but that its population recovers as the p -state relaxes. The existence of an intermediate state II is inferred from the observation that ground-state recovery is noticeably slower than decay of the blue curve. With reference to Fig. 1, state II can be assigned either to p -state signal subsequent to solvent relaxation in the excited state, or to s -state signal just after IC, in which the electron is surrounded by a vibrationally excited, nonequilibrium distribution of solvent molecules. This assignment determines whether the decay of the blue signal in Fig. 4 corresponds to relaxation within the p -state or to IC to the s -state.

The latter assignment is supported by several factors. First, as shown in Fig. 3A, the energy interval corresponding to feature II falls on the high-eKE edge of the ground-state spectrum but clearly lies within the eKE range of the ground state, just where one would expect to see a contribution from vibrationally hot s -state signal. A close examination of Fig. 3B suggests that for $\Delta t_{12} \geq 230$ fs, the pump-induced signal shifts toward lower eKE as it loses intensity, as one might expect for signal associated with solvent relaxation on the ground state.

Moreover, assigning the red signal to the relaxed p -state signal would indicate an energy shift of around -0.8 eV within the p -state subsequent to photoexcitation, as seen from the difference between the blue and red energy windows and the data in Fig. 3B. Quantum-classical molecular dynamics simulations of hydrated electron pump-probe signal using various electron-water pseudopotentials do not support such a large energy shift (6, 14); instead, they find the p -state energy remains nearly constant, whereas the s -state energy exhibits large fluctuations. It thus appears more reasonable to attribute the decay of the blue signal I to IC, and the decay (recovery) of the red (green) signal to solvent relaxation on the s -state subsequent to IC. This assignment then yields $\tau_1 = \tau_{IC} = 75 \pm 20$ fs, whereas $\tau_2 = \tau_s = 410 \pm 40$ fs represents the time constant for solvent relaxation subsequent to IC.

Our value of 75 ± 20 fs for τ_{IC} supports the nonadiabatic relaxation mechanism shown in Fig. 1, apparently resolving the ambiguity from the TA experiments (which yielded similar time constants). Moreover, this value is remarkably consistent with the extrapolated τ_{IC} of 60 fs obtained from size-selected water cluster anions, suggesting that both the relaxation dynamics and the VDEs for water cluster anions can be extrapolated to bulk values. The data as interpreted here also yield a measurement of the VDE for the p -state of the hydrated electron, 2.2 ± 0.2 eV, taking the center of the eKE distribution in Fig. 3C to be 2.5 eV.

We next considered whether our results show any evidence for p -state relaxation. Our more recent work on water cluster anions showed the p -state photoelectron signal shifting to lower eKE on the same time scale as IC, implying that these two processes occur in parallel (18). Similar dynamics are suggested here by Fig. 3C, which shows the early-time signal shifting toward lower eKE as it disappears. However, this p -state signal overlaps the hot s -state signal, which is shifting in the same direction, so the trend in Fig. 3C should be viewed with caution. We do not see evidence for the slowest time constant (1.1 ps) seen in the TA experiments, possibly because relatively few data points were taken at long time delays.

The experiments presented here open up the possibility of tracking solvated electron dynamics in methanol and other solvents, in which similar mechanistic issues have been raised from TA experiments (35). Furthermore, with differing excitation schemes (36) it should be possible to probe the relaxation dynamics of prehydrated and conduction-band electrons in liquid water jets. These electrons are more highly excited and delocalized than are the p -state electrons probed here, and their energetics and dynamics have been investigated but are not as well-characterized (37–39). Hence, TRPES in liquid jets offers considerable potential for unraveling electron dynamics in water and other solvents.

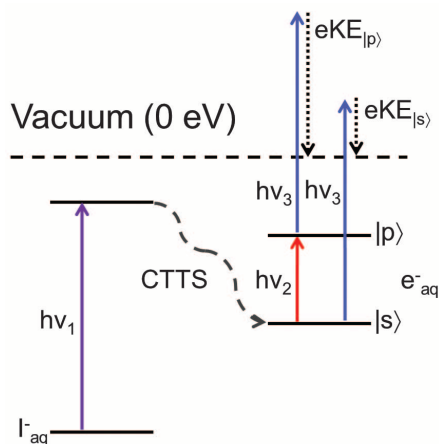


Fig. 2. Energy diagram of experiment. Three femtosecond laser pulses interact with the liquid jet.

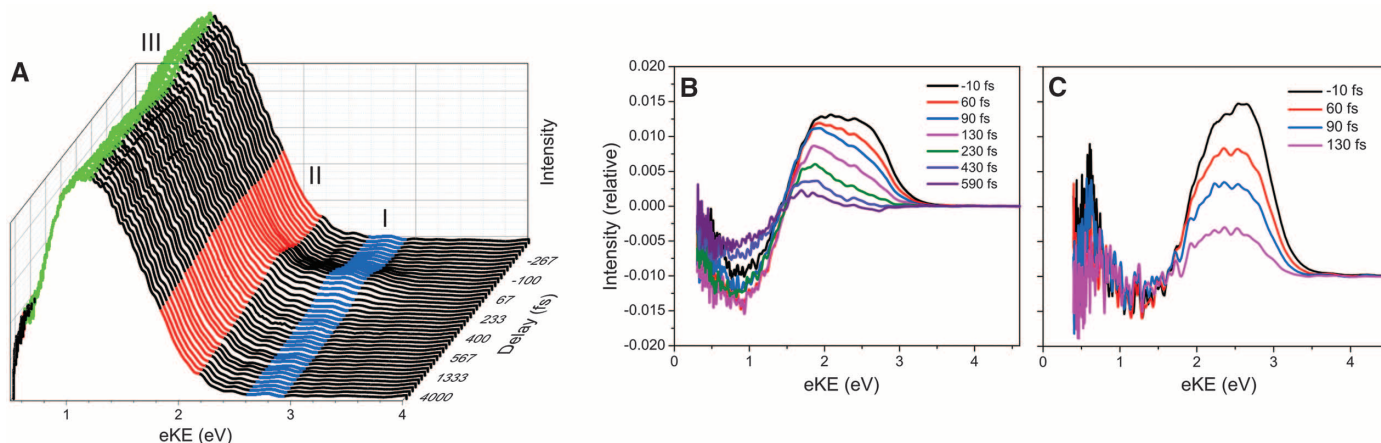
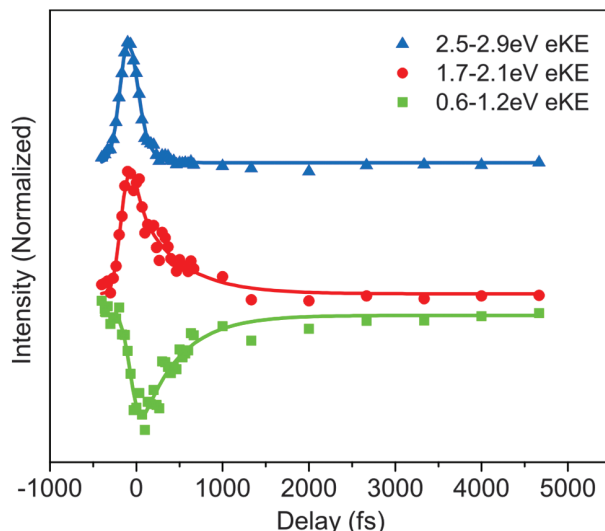


Fig. 3. Time-resolved photoelectron spectra. The plots show three-pulse spectra from which (A) the contribution from each one color spectrum has been subtracted and (B) the $(h\nu_1 + h\nu_3)$ two-color spectrum has been subtracted. (C) Spectra from (B) at early times, showing curve at 167 fs subtracted.

Fig. 4. Integrated intensity as a function of delay over three eKE intervals. Colors are the same as in Fig. 3A.



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Materials and Methods

Supplementary Text

Fig. S1

Reference (40)

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Dynamical Resonances Accessible Only by Reagent Vibrational Excitation in the $F + HD \rightarrow HF + D$ Reaction

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Experimental limitations in vibrational excitation efficiency have previously hindered investigation of how vibrational energy might mediate the role of dynamical resonances in bimolecular reactions. Here, we report on a high-resolution crossed-molecular-beam experiment on the vibrationally excited $HD(v=1) + F \rightarrow HF + D$ reaction, in which two broad peaks for backward-scattered $HF(v'=2$ and 3) products clearly emerge at collision energies of 0.21 kilocalories per mole (kcal/mol) and 0.62 kcal/mol from differential cross sections measured over a range of energies. We attribute these features to excited Feshbach resonances trapped in the peculiar $HF(v'=4)-D$ vibrationally adiabatic potential in the postbarrier region. Quantum dynamics calculations on a highly accurate potential energy surface show that these resonance states correlate to the $HD(v'=1)$ state in the entrance channel and therefore can only be accessed by the vibrationally excited HD reagent.

Molecular vibrations have profound effects on chemical reactivity. Early studies on atom-diatom reactions led to the establishment of the Polanyi rules, which state that vibrational energy is more efficient than translational energy in promoting a late-barrier reaction,

whereas the reverse is true for an early barrier reaction (*1*). Crim, Zare, and co-workers later observed great enhancements of reactivity in the late-barrier $H + H_2O/HOD/D_2O$ reactions by reagent vibrational excitation (2–5), based on theoretical predictions made by Schatz and co-workers

(6). More recently, experimental and theoretical studies have explored the generality and validity of the rules for polyatomic systems involving CH_4 and its isotopically substituted analogs (7–15). Most notably, Liu and co-workers carried out extensive experimental investigations on the dynamics of the $F/Cl + CHD_3$ reactions with preliminary CH stretching excitation, and their unexpected observations presented strong challenges to theory (10–15).

Reactive resonances, transiently trapped quantum states along the reaction coordinate in the transition-state region of a chemical reaction, have also occupied a central place in reaction dynamics research over the past few decades (16–19), particularly in the context of $F + H_2/HD$ reactions (20–29). Recent experimental studies, in combination with quantum dynamics calculations on a

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highly accurate potential energy surface (PES), revealed that the course of the $F + HD(v = 0)$ reaction is dominated by a dynamical resonance trapped in the peculiar $HF(v' = 3)$ -D vibrationally adiabatic potential (VAP) well at the low-collision energy region, where v and v' represent the vibrational quantum number for the reactant and product molecule, respectively (27–29). As the collision energy increases, the reaction trajectory eventually encounters the $HF(v' = 4)$ -D VAP at the product side. However, theoretical studies on the highly accurate PES reveal that there is no resonance signal for the reaction channel in higher-energy collision regions, raising the intriguing question of whether there are any reactive resonances in the high-energy region, trapped in the $HF(v' = 4)$ -D VAP well. If such resonances exist, how can we probe them properly?

We report here on a combined crossed-molecular-beam experimental and theoretical study on the $F + HD(v = 1)$ reaction to probe the possible dynamical resonances in the high-energy region of the reaction using a vibrationally excited reagent. Molecular vibrational states of infrared (IR) active modes can be efficiently populated by direct excitation using strong narrow-bandwidth IR lasers. In the case of nonpolar molecules such as H_2 with IR-inactive modes, stimulated Raman pumping (SRP) has been the

method of choice for preparing excited vibrational states. Product state distribution has been measured for the $D + H_2(v = 1)$ reaction using the SRP pumping scheme (30). However, the lower excitation efficiency of SRP has thus far limited its application for crossed-molecular-beam experiments. Recently, Mukherjee and Zare proposed a scheme of Stark-induced adiabatic Raman passage (SARP) (31), an important extension of adiabatic passage techniques (32) to excite Raman-active vibrations using a virtual intermediate state. Following this idea, highly efficient excitation of H_2 and HD from $v = 0$ to $v = 1$ has been demonstrated in two laboratories using state-of-the-art high-power nanosecond lasers (33, 34). However, because the SRP scheme required both the high-power pump and Stokes lasers to be tightly focused (~ 0.2 by 0.2 mm^2) to achieve near-unity pumping efficiency, the prepared $HD(v = 1)$ population throughout the crossed-beams region (~ 3 by 3 mm^2) was insufficient for crossed-molecular-beam experiments. We have therefore explored the SRP pumping conditions by using defocused pump and Stokes lasers to cover the whole crossed region and found that overall higher population of $HD(v = 1)$ can be achieved, thereby enabling a high-resolution crossed-beam experiment on the $F + HD(v = 1)$, using the D-atom Rydberg tagging technique. (See more details in the supplementary materials.)

Time-of-flight (TOF) spectra of the D atom products from the $F + HD(v = 0 \text{ and } 1)$ reaction, with the Raman Stokes laser on and off, were measured at laboratory angles varied in 10° intervals at collision energies E_c of 0.23 and 0.63 kcal/mol (Fig. 1). Thanks to the high resolution of the Rydberg tagging technique, the spectra for the $F + HD(v = 1, j = 0)$ reaction can be reliably

obtained by subtracting the spectra with the Stokes laser off from those with the Stokes laser on, where j is the rotational quantum number for the reactant molecule. The main structures in the resulting TOF spectra can be clearly assigned to the HF product rovibrational states from the $HD(v = 1)$ reaction. The obtained spectra were then converted to the center-of-mass frame to obtain the product kinetic energy distributions, from which full rovibrational state-resolved differential cross section (DCS) values were determined for these two collision energies (Fig. 2, A and B).

For both collision energies, the HF product was produced mainly in the $v' = 3$ state, in strong contrast with the $F + HD(v = 0)$ reaction, which produces HF mainly in $v' = 2$. At $E_c = 0.23$ kcal/mol, the product HF is largely backward-scattered with respect to the F-atom beam direction, with some very small forward-scattering components. At $E_c = 0.63$ kcal/mol, forward-scattering peaks appear for both the HF $v' = 2$ and 3 products, although in much more pronounced form for $v' = 3$, implying that there may be reactive resonances for the reaction.

To provide additional clues for the possible reactive resonances, we also carried out a careful measurement of the collision energy dependence of the DCS for the HF($v' = 2$ and 3) product in the backward-scattering direction with the Stokes laser on and off. The detector was fixed in the backward direction at different collision energies, and the measurement was repeated 10 times at different collision energies to reduce experimental error, which was estimated to be about 10%. The Stokes laser-off measurements reproduced the energy dependence of DCS in the backward direction for the $F + HD(v = 0) \rightarrow HF(v' = 1 \text{ and } 2) + D$ reaction that

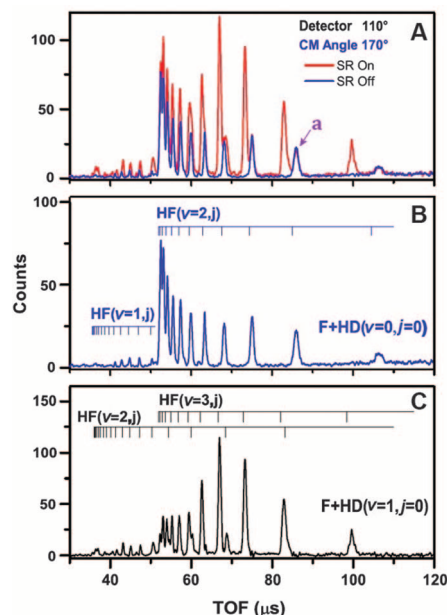


Fig. 1. (A to C) TOF spectra of the D atom product from the $F + HD \rightarrow HF + D$ reaction at a collision energy of 0.23 kcal/mol. The laboratory angle is 110° , which corresponds to 170° in the center-of-mass frame. The blue and red lines in (A) indicate measurements with the stimulated Raman (SR) laser pulse on and off, respectively. The counts ratio between SR on and off can be determined by the peak marked “a” in (A). Consequently, the TOF spectra produced by the vibrational excited state HD can be accordingly extracted, as shown in (C), in contrast with that for the ground state HD shown in (B).

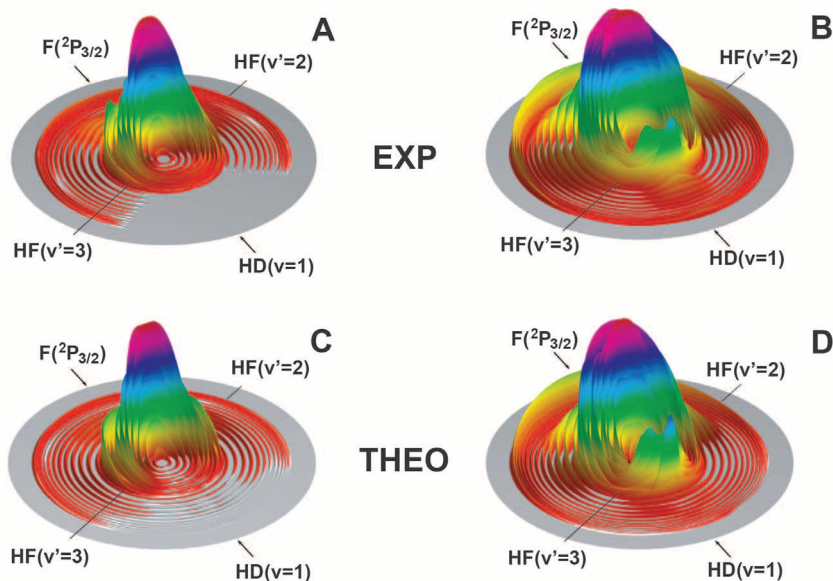


Fig. 2. Three-dimensional product contour plots as a function of product velocity. Product contour plots versus product velocity are shown in the center-of-mass frame for [(A) and (B)] experimental measurements and [(C) and (D)] theoretical calculations at collision energies of [(A) and (C)] 0.23 kcal/mol and [(B) and (D)] 0.63 kcal/mol.

we obtained in previous studies (27), in which a single resonance peak at 0.4 kcal/mol was clearly observed. The data for the $F + HD(v = 1)$ reaction in the collision energy range of 0.2 to 0.8 kcal/mol, however, exhibit two broad peaks

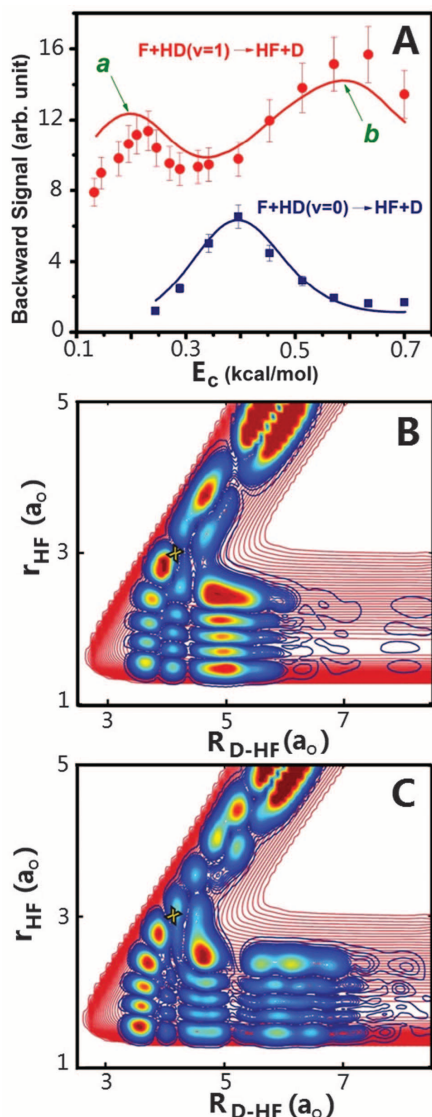


Fig. 3. Collision energy dependence of the DCS. (A) Theoretical (solid lines) and experimental (solid circles and squares) DCS for the backward-scattering HF product of $F + HD(v = 1, j = 0)$ (red line and full circle) and the HF product of $F + HD(v = 0, j = 0)$ (blue line and full square), over a range of collision energies E_c . The backward-scattering product signals (DCS) at different collision energies were measured by varying the collision energy back and forward 10 times and then summed over. The error bars shown in this figure were estimated by analyzing the signal fluctuations in these scans and should be taken as ± 1 SD of uncertainty. (B) and (C) present the reactive resonance wave functions (204) and (304), which enhance the reaction at collision energies of 0.20 and 0.66 kcal/mol with reactant $HD(v = 1, j = 0)$. The contour lines in red in (B) and (C) are the corresponding 2D PES, and the yellow cross represents the transition-state point.

for the HF ($v' = 2$ and 3) products, at collision energies of 0.21 (peak a) and 0.62 (peak b) kcal/mol, respectively (Fig. 3A).

To interpret the experimental observation, we improved the Fu-Xu-Zhang (FXZ) PES for the reaction system by adding higher-level ab initio data points to more accurately reproduce the features around the transition barrier (see the supplementary materials). Extensive studies showed that the new PES, without any scaling factor to the original ab initio energies, is better than the FXZ PES in describing the various dynamical processes in the system. Theoretical three-dimensional (3D) polar DCSs obtained on the new PES at collision energies of 0.23 and 0.63 kcal/mol reproduce remarkably well the corresponding experimental counterparts (Fig. 2, C and D). The energy dependence of the backward-scattered HF ($v' = 2$ and 3) calculated on the new PES also agrees well with the experimental measurement, despite the fact that the relative peak heights and positions are slightly different from the experiment (Fig. 3A). It is found that the backward-scattered resonance peaks are mainly contributed by $J = 0$ to 10 partial waves, where J is the total angular momentum of the reaction system (fig. S1). The calculations also reveal that the integral cross sections (ICS) for product HF ($v' = 2$ and 3) as a function of collision energy show two similar broad peaks around collision energies of 0.25 and 0.75 kcal/mol (fig. S2). The peaks in the backward-scattering signal and ICS as a function of collision energy are found to have the same dynamical origin.

The energy dependence of the total reaction probability for the total angular momentum $J = 0$ exhibits two distinct peaks, at 0.20 (A state) and 0.66 (B state) kcal/mol, which correspond to two reactive resonance states (fig. S3). Time-dependent wave-packet calculations were performed to extract the exact scattering wave functions at these two collision energies for $J = 0$. The 3D wave function at the collision energy of 0.20 kcal/mol shows the existence of four nodes along the H-F coordinate (correlating to the HF product) in the

HF-D complex and two nodes along the reaction coordinate, with the outgoing wave function mainly in the HF ($v' = 3$) state (Fig. 3B). Therefore, the resonance state A is the second excited state trapped in the HF ($v' = 4$)-D vibrational adiabatic potential (VAP) well, which decays mostly to produce HF ($v' = 3$) product. It can be assigned as ($v_1 = 2, v_2 = 0, v_3 = 4$) or (204) state for the reaction complex $FHD^\ddagger$, where v_1 is the quantum number for the D-HF stretching mode, v_2 for the bending mode, and v_3 for the HF stretching mode. The 3D scattering wave function for $J = 0$ at the collision energy of 0.66 kcal/mol has a similar structure along the H-F coordinate as state A, but with three nodes along the reaction coordinate, hence corresponding to the third excited resonance state in the HF ($v' = 4$) VAP well, i.e., the (304) state (Fig. 3C). Using the spectral quantization method (35), we found that there is another resonance state with an energy ~ 2.26 kcal/mol below the $HD(v = 1)$ asymptote, with four nodes along the H-F coordinate and one node along the reaction coordinate (fig. S4), which can be assigned as the (104) resonance state and is inaccessible by the $F + HD(v = 1)$ reaction channel.

Wave functions presented in Fig. 3, B and C and fig. S4 clearly show that the resonances decay mostly into the $HD(v = 1)$ state in the entrance channel, indicating that these resonance states are trapped in a vibrationally adiabatic well formed by the HF ($v' = 4$)-D VAP in the product side correlating to the $HD(v = 1)$ state in the entrance channel (Fig. 4). Reaction trajectories with $HD(v = 0)$ can only explore the right side of the $v' = 4$ well. By adding the same amount of energy of one quantum HD vibration to the translational motion of the $F + HD(v = 0)$ reagents, the reaction can only take place on the $v = 0$ reaction pathway and, therefore, cannot access these resonance states. Indeed, quantum scattering calculations of the same PES with the same total energy (all in translation) as the $F + HD(v = 1)$ reaction do not show any evidence of resonances (see the supplementary materials). This clearly reveals that initial vibrational

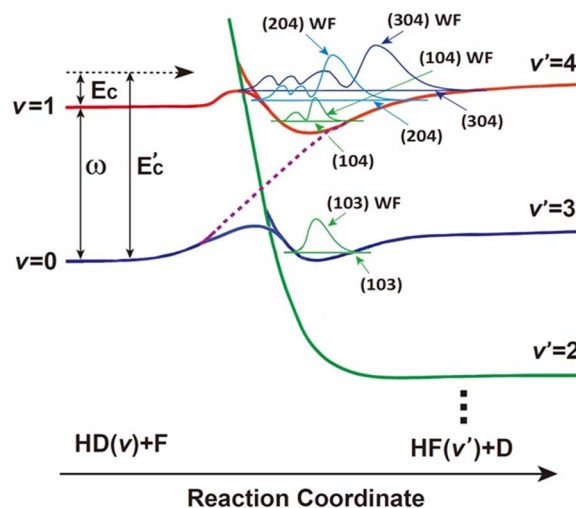


Fig. 4. Schematic diagram showing the resonance-mediated reaction mechanism for the $F + HD \rightarrow HF + D$ reaction. Three resonance states are trapped in the peculiar HF ($v' = 4$)-D VAP well. The 1D wave functions of the resonance states are also shown. The (104), (204), and (304) states are the first, second, and third excited resonance state, respectively. Only reactant $HD(v = 1)$ can effectively correlate with the resonance states (204) and (304).

excitation not only provides energy required for the reaction but also gives rise to a proper adiabatic pathway that accesses the dynamical resonances in the high-energy region, whereas translational energy cannot provide such a pathway. The results presented here show that vibrationally excited molecular reactions provide a unique context for probing dynamical resonances in chemical reactions when the vibrationally adiabatic potential, which correlates to the vibrationally excited state of the reagent in the entrance channel, supports quasibound resonance states.

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Supplementary Materials

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Supplementary Text
Figs. S1 to S6
References (36–39)

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Unexpected Stable Stoichiometries of Sodium Chlorides

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Sodium chloride (NaCl), or rocksalt, is well characterized at ambient pressure. As a result of the large electronegativity difference between Na and Cl atoms, it has highly ionic chemical bonding (with 1:1 stoichiometry dictated by charge balance) and B1-type crystal structure. By combining theoretical predictions and diamond anvil cell experiments, we found that new materials with different stoichiometries emerge at high pressures. Compounds such as Na₃Cl, Na₂Cl, Na₃Cl₂, NaCl₃, and NaCl₇ are theoretically stable and have unusual bonding and electronic properties. To test this prediction, we synthesized cubic and orthorhombic NaCl₃ and two-dimensional metallic tetragonal Na₃Cl. These experiments establish that compounds violating chemical intuition can be thermodynamically stable even in simple systems at nonambient conditions.

At ambient conditions, NaCl is the only known stable compound in the Na-Cl system, the chemistry of which is well

understood. Extreme conditions, such as high pressure, change the chemical properties of the elements, including Na and Cl (1, 2), and the pressure × volume term in the free energy becomes greater than energy of chemical bonds. Thus, strongly compressed matter may exist in totally counterintuitive chemical regimes. Although unexpected high-pressure phenomena may be present in planetary systems or lead to novel exotic materials, we still lack a consistent fundamental understanding of even seemingly simple binary systems such as Na-Cl.

The high-pressure behavior of NaCl has been extensively studied experimentally at pressures up to 304 GPa (3–6) and by ab initio simulations (7–9), and very simple behavior was observed: At 30 GPa, the rocksalt structure was found to transform into the CsCl (B2-type) structure (6, 8). From the Herzfeld criterion, metallization of NaCl is expected to occur at 300 GPa (10), whereas density functional theory calculations (9) suggest 584 GPa.

To find stable Na-Cl compounds and their structures that may not have been possible to observe experimentally or computationally, we used the ab initio evolutionary algorithm USPEX (11–14), which can simultaneously find stable stoichiometries and the corresponding structures in multicomponent systems (15). In these calculations, any combinations of numbers of atoms in the unit cell were allowed (with the total number ≤16), and calculations were performed at pressures of 1 atm, 20 GPa, 100 GPa, 150 GPa, 200 GPa, and 250 GPa. Detailed enthalpy calculations for the most stable structures allowed us to reconstruct the *P*-*x* phase diagram of the Na-Cl system (Fig. 1 and figs. S1 and S2).

To verify these predictions, we performed high-pressure experiments in a laser-heated diamond anvil cell at 10 to 80 GPa on the Na-Cl system in the presence of excess chlorine and sodium (15). We specifically targeted the synthesis of Na₃Cl and NaCl₃, which were predicted to become stable at the lowest pressures. The reaction products were examined by visual observations, optical absorption spectroscopy and Raman confocal spectroscopy, and by synchrotron x-ray diffraction (XRD) probes at room temperature (15).

The calculated phase diagram features unexpected compounds: NaCl₃, stable above 20 GPa; NaCl₇, stable above 142 GPa; and Na₃Cl₂, Na₂Cl, and Na₃Cl, which are stable above 120 GPa, 100 GPa, and 77 GPa, respectively. We define as stable those compositions that have lower free energy than any isochemical mixture of other compounds or pure elements. For all the newly predicted structures we computed phonons, and found them to be dynamically stable. In the entire region of explored pressures, NaCl is also a stable compound; it will not spontaneously decompose into other compounds. Thus, to obtain the newly predicted compounds, it is not sufficient merely to

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compress NaCl; one must do so at high temperatures (to overcome kinetic barriers) and with excess of either Na or Cl. Electronic densities of states (fig. S5) show that most of these compounds are poor metals, with pronounced pseudogaps at the Fermi level. Cl-rich compounds can be considered as n-type semiconductors, whereas Na-rich phases are p-type semiconductors. Pseudogaps imply an electronic mechanism of their stabilization.

At 20 to 48 GPa, NaCl_3 is stable in the *Pnma* structure, which has four formula units in the unit cell. Unlike all the other new phases predicted here (which are metallic), this phase is a semiconductor. Its structure (Fig. 2B) contains almost linear asymmetric Cl_3 groups. Bader analysis (16) shows that the middle atom in the Cl_3 group is nearly neutral, with most negative charge on the side atoms (table S1) and the total charge of this anion group is ~ -0.8 . *Pnma*- NaCl_3 has ionic bonding between Na^+ and $[\text{Cl}_3]^-$ and rather unusual covalent bonding within $[\text{Cl}_3]^-$ groups. The latter are reminiscent of the well-known trihalide ions I_3^- , Br_3^- , and ClICl^- , and of the hypothetical H_3^- ion (17), which is predicted to have charge configuration $[\text{H}^{-0.81}\text{H}^{+0.72}\text{H}^{-0.81}]^{-0.9}$. In a Zintl-like scheme, the central Cl atom must be positively charged to be able to form two covalent bonds and satisfy the octet rule. In the valence-shell electron pair repulsion model (18), the central Cl atom of the Cl_3^- group adopts the dsp^3 hybridization and has five electron pairs, bringing a negative net charge and violating the octet rule. For chlorine atoms, unlike iodine, it is not easy to populate vacant d orbitals (which nicely explains the structure of ClICl^- ions) and the two schemes work simultaneously; this explains the nearly zero charge of this central atom and its increased (although still relatively small) d-orbital population. At 48 GPa, this peculiar insulating ionic state breaks down, and NaCl_3 transforms into a metallic A15 (Cr_3Si -type) structure with space group *Pm3n*.

NaCl_7 , stable above 142 GPa, has a cubic structure (space group *Pm3*; Fig. 2A) derivative of the A15 ($\beta\text{-W}$ or Cr_3Si) type. The *Pm3*- NaCl_7 structure is obtained from *Pm3n*- NaCl_3 by substituting the central Na atom (inside a Cl_{12} icosahedron) with Cl (Fig. 2, A and C). The lattice parameters and bond lengths of NaCl_3 and NaCl_7 are very close (e.g., within 0.5% at 200 GPa) because at this pressure Na and Cl have almost identical sizes, whereas in ambient conditions Cl^- is much larger than Na^+ (ionic radii are 1.81 Å and 1.02 Å, respectively). This opens the possibility of non-stoichiometry and disorder, with the potential for Anderson localization of electronic and phonon states.

At 200 GPa, the shortest Cl-Cl distance in NaCl_3 is 2.06 Å, only slightly longer than the Cl-Cl bond in the Cl_2 molecule (1.99 Å). These Cl-Cl bonds form extended monatomic chains running along the three mutually perpendicular axes. This type of structure resembles a textbook linear chain with a partially filled band, which in the free state is unstable against Peierls distortion (19). An isolated Cl chain has a $\frac{1}{2}$ filled band, and at low pressures it should break into Cl_2 molecules, but in NaCl_3 this band is $\frac{2}{3}$ filled as a result of the extra electron donated by Na, and the chain should break into Cl_3^- ions, which we indeed see in the *Pnma*- NaCl_3 phase at lower pressures. The application of pressure and the influence of other chemical entities (Na and non-chain Cl atoms) stabilize these chains in NaCl_3 , NaCl_7 , and elemental chlorine. Peierls theorem also explains the results of our phonon calculations indicating that both *Pm3n*- NaCl_3 and NaCl_7 can exist only at high pressure and are not quenchable to atmospheric pressure.

Electronic band structures of NaCl_3 and NaCl_7 show a deep and wide pseudogap for NaCl_3 -*Pm3n* (Fig. 3, A and B). In both structures, Cl atoms forming the Cl_{12} icosahedra show toroidal

electron localization function (ELF) maxima (Fig. 3, C and D) corresponding to a non-closed-shell electronic configuration, whereas the Cl1 atom occupying the center of the Cl_{12} icosahedron in NaCl_7 has a spherical ELF maximum. Thus, Cl1 and Cl2 atoms in NaCl_7 have different electronic structures and play very different chemical roles. Bader analysis (table S1) confirms this and gives an unusual positive charge to the Cl1.

For the Na-rich side of the phase diagram, we predict several thermodynamically stable compounds: tetragonal Na_3Cl (space group *P4/mmm*), two phases of Na_3Cl_2 [tetragonal (space group *P4/m*) and orthorhombic (space group *Cmmm*)], and three phases of Na_2Cl —one tetragonal (space group *P4/mmm*) and two orthorhombic (space groups *Cmmm* and *Imma*). Most of these are layered superstructures of the CsCl-type (B2) structure, with both Na and Cl atoms in the eight-fold coordination (Fig. 2). For example, Na_3Cl can be represented as a $[\text{NaCl}][\text{Na}_2][\text{NaCl}][\text{Na}_2]\dots$ sequence of layers, and the *c* parameter of the unit cell is doubled relative to that of B2-NaCl. This and similar structures have very interesting 2D metallic features, with alternating metallic $[\text{Na}_2]$ and insulating $[\text{NaCl}]$ layers. Na_3Cl_2 is stable above 120 GPa, and its *P4/m* structure can be described as a 1D-ordered (rather than layered, 2D-ordered) substitutional superstructure of the B2-NaCl structure. Compound Na_2Cl shows a more complex behavior than Na_3Cl or Na_3Cl_2 . At 100 to 135 GPa, the *P4/mmm* structure is stable; it is also a layered B2-type superstructure. Na_2Cl is stable at 135 to 298 GPa in the *Cmmm* structure, and above 298 GPa in the *Imma* structure, with Na and Cl atoms in the 12-fold coordination in the former, and 10- and 12-fold coordination, respectively, in the latter structure (Fig. 2, H and I).

XRD measurements of Na_3Cl and NaCl_3 synthesized at high pressures show new Bragg peaks after laser heating. At pressures above 60 GPa,

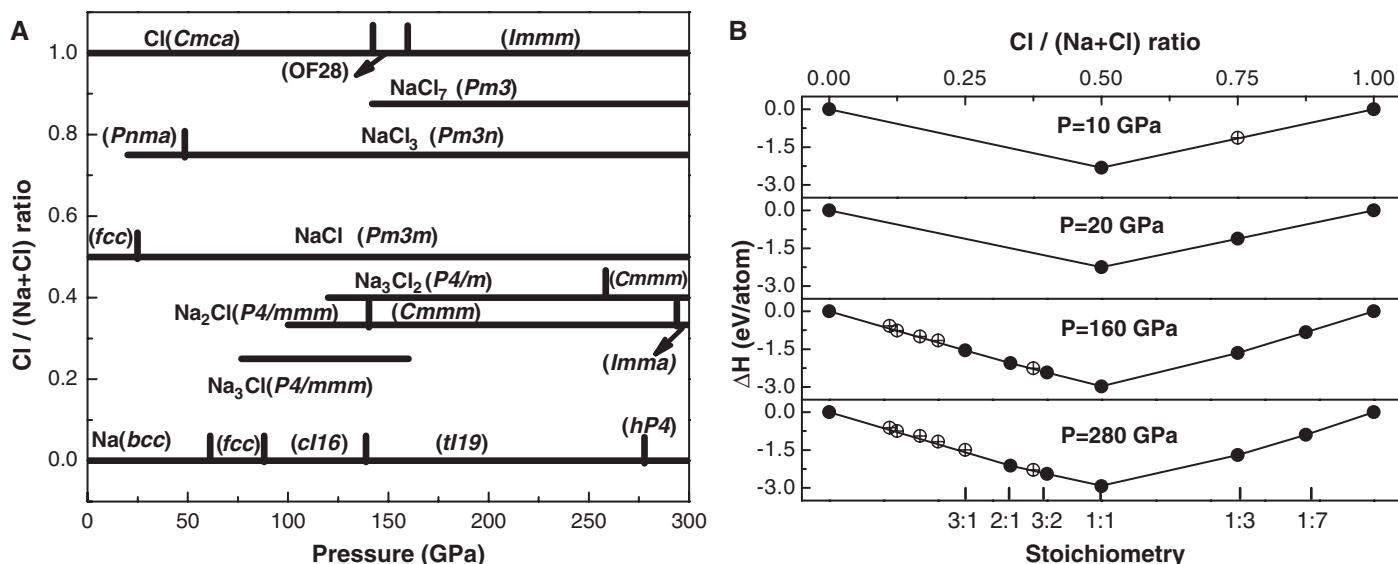


Fig. 1. Stability of new sodium chlorides. (A) Pressure-composition phase diagram of the Na-Cl system. (B) Convex hull diagram for Na-Cl system at selected pressures. Solid circles represent stable compounds; open circles denote metastable compounds. ΔH denotes the enthalpy of formation per atom.

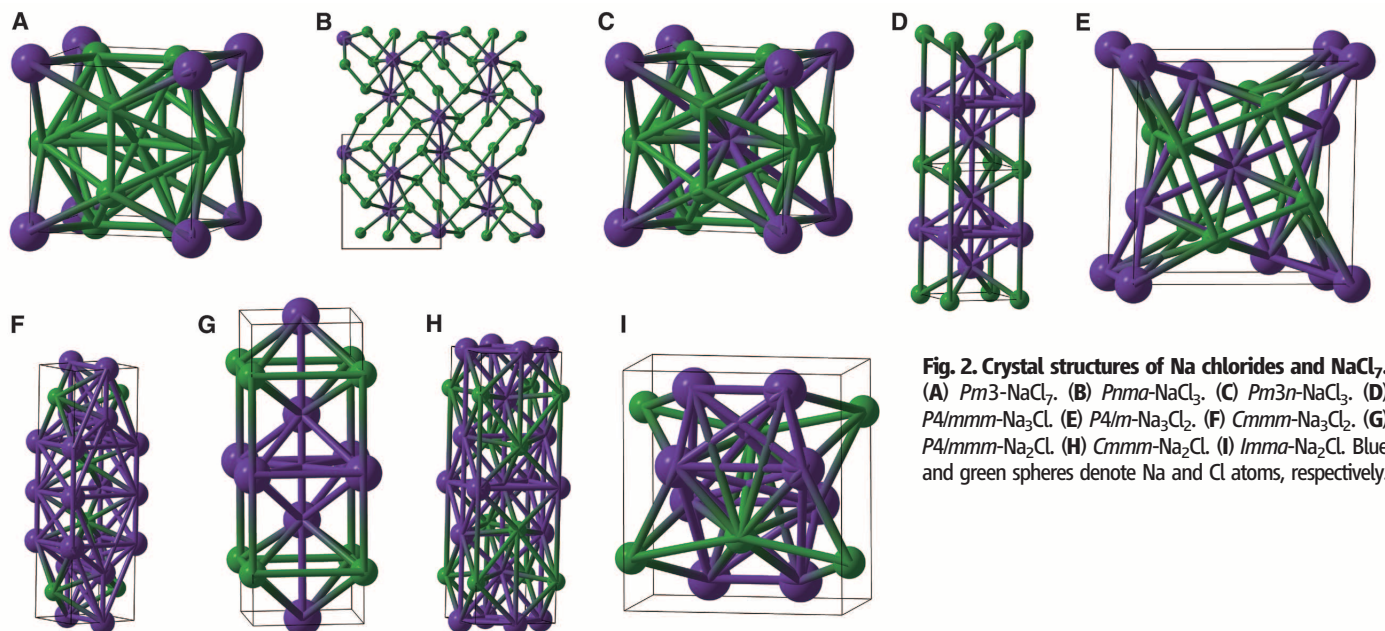


Fig. 2. Crystal structures of Na chlorides and NaCl_7 . (A) $Pm\bar{3}$ - NaCl_7 . (B) $Pnma$ - NaCl_3 . (C) $Pm\bar{3}n$ - NaCl_3 . (D) $P4/mmm$ - Na_3Cl . (E) $P4/m$ - Na_3Cl_2 . (F) $Cmmm$ - Na_3Cl_2 . (G) $P4/mmm$ - Na_2Cl . (H) $Cmmm$ - Na_2Cl . (I) $Imma$ - Na_2Cl . Blue and green spheres denote Na and Cl atoms, respectively.

these Bragg peaks can be indexed either in a cubic NaCl_3 unit cell (Fig. 4A) or with a mixture of the cubic and the orthorhombic $Pnma$ NaCl_3 unit cell. With pressure decreasing below 54 GPa, after laser heating, only the peaks of the orthorhombic NaCl_3 are present in the XRD patterns (fig. S7). The XRD pattern usually also contains peaks from unreacted B2- NaCl and orthorhombic chlorine, which were identified using our theoretical calculations.

From the XRD data, we obtained the lattice parameters and the unit cell volume for the two structures as a function of pressure for the decompression sequence. There is good agreement between the experimental and theoretical equations of state for both NaCl_3 structures (Fig. 4C). Also, in agreement with the theoretical predictions, we find that two new NaCl_3 phases transform from one to the other upon pressure release at 300 K, although the transition is sluggish and there is a large range of phase coexistence. $Pnma$ - NaCl_3 remains metastable at pressures as low as 18 GPa and decomposes to NaCl and Cl_2 at lower pressures.

Raman spectroscopy of reacted Cl-rich compounds confirmed the XRD data. We observed Raman spectra of two different kinds; at some pressure and observation points, they were even superimposed. The Raman spectra of the excess Cl_2 were easy to identify because we also collected reference data for unreacted materials as a function of pressure. The Raman spectra of two polymorphic modifications of NaCl_3 (figs. S8 and S9) are quite different. In cubic NaCl_3 we observe one broad strong band near 450 cm^{-1} and a number of weaker features, whereas in $Pnma$ NaCl_3 there are a number of narrow peaks. In both cases the agreement between theory and experiment is good concerning the major peak positions. Moreover, experimental and theoretical pressure dependences of the Raman frequencies (fig. S10) are

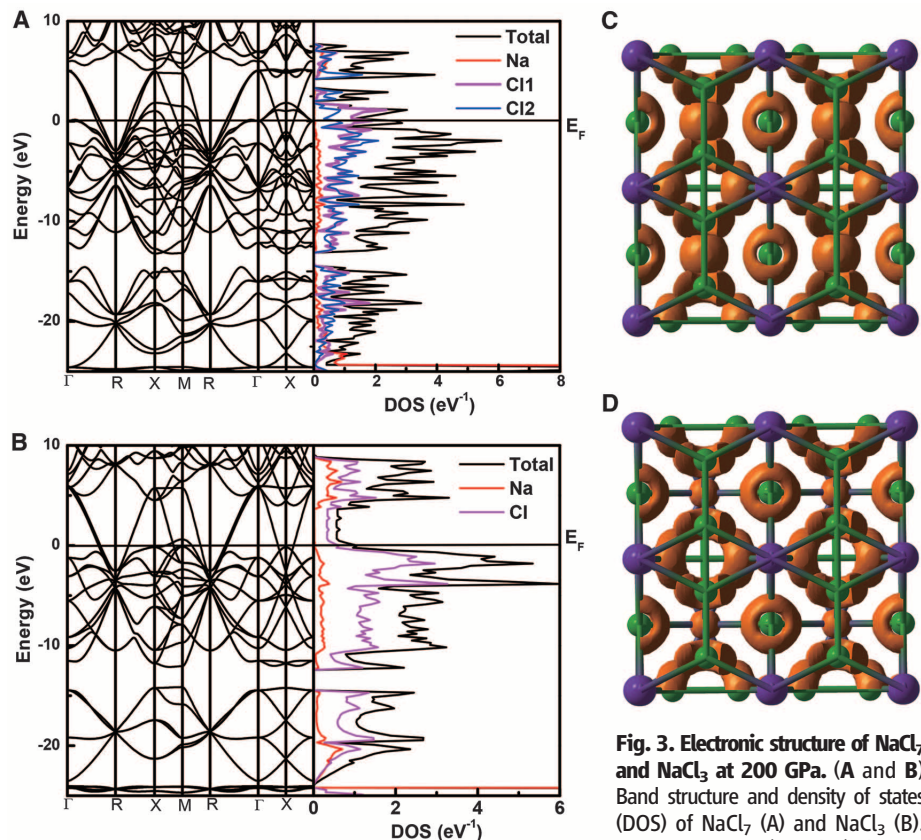


Fig. 3. Electronic structure of NaCl_7 and NaCl_3 at 200 GPa. (A and B) Band structure and density of states (DOS) of NaCl_7 (A) and NaCl_3 (B). E_F , Fermi energy. (C and D) Electron localization function of (C) NaCl_7 and (D) NaCl_3 at $\text{ELF} = 0.80$. For clarity, atom-projected DOSs in (A) and (B) were multiplied by 3 (for NaCl_7) and by 4 (for NaCl_3). Colors are as in (A) and (B).

also in good agreement for both structures. However, in cubic phase we observed a number of extra peaks (Raman-forbidden for a $Pm\bar{3}n$ lattice) corresponding to other zone-center phonons; that is, some of the selection rules appear to be

lifted. These selection rules could be substantially lifted in surface Raman scattering (as happens in metals), because of disorder in site occupation or variable stoichiometry (as Cl and Na are easily interchangeable at high pressure). Optical

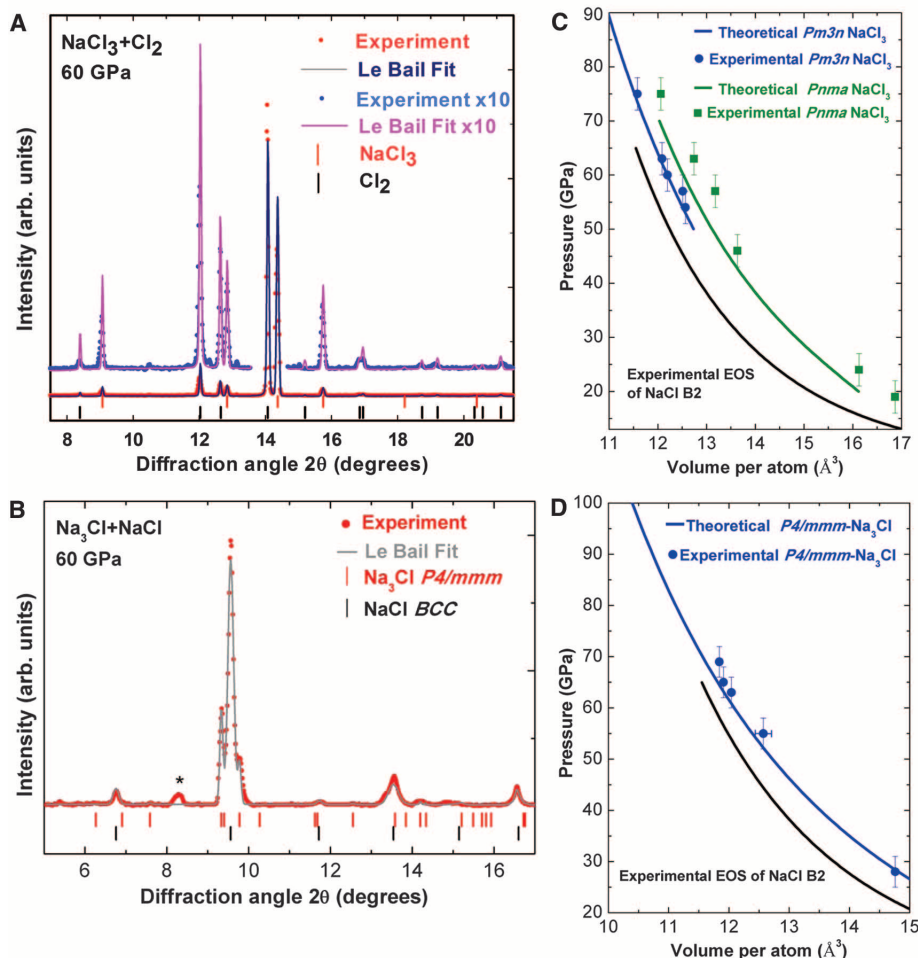


Fig. 4. Powder x-ray diffraction patterns and equations of state for NaCl_3 and Na_3Cl . (A and B) NaCl_3 in Cl_2 medium (A) and Na_3Cl in Na medium (B) collected at 60 GPa. Vertical ticks correspond to Bragg peaks of Na-Cl phases. The x-ray wavelengths are 0.5146 Å in (A) and 0.3344 Å in (B). The peak marked by the asterisk corresponds to the strongest peak of rhenium (gasket material). (C and D) Equations of state of NaCl_3 (C) and Na_3Cl (D) as determined experimentally (symbols) and theoretically (lines).

absorption spectra of the synthesized material (fig. S6) show a gap-like feature at 1.7 eV, which is consistent with the predicted prominent pseudogap in the electronic density of states (Fig. 3B).

The case of Na-rich material is similar but less complex. XRD of the samples laser-heated above 60 GPa showed new Bragg peaks that could be indexed in a tetragonal $P4/mmm$ unit cell (Fig. 4B) across the whole pressure range of this study. The XRD pattern usually also contained peaks from unreacted cubic B1 or B2 NaCl and body-centered cubic (bcc) or face-centered cubic (fcc) sodium (20). The lattice parameters of new material agree well with the theoretically predicted $P4/mmm$ Na_3Cl in a wide pressure range of 27 to 70 GPa (Fig. 4D). The Raman data (fig. S11) are also consistent with theoretical predictions. However, as in the case of the Cl-rich compounds, in addition to two Raman-allowed modes, we also observe a number of Raman-forbidden bands whose positions agree reasonably well with other computed zone-center phonons. Finally, Raman data show that newly synthesized Na-rich material can be (meta)

stable down to 20 GPa (figs. S11 and S12) and then decomposes into NaCl and Na at lower pressures.

The theoretical prediction and experimental synthesis of unexpected chemical compounds in a simple binary system, such as Na-Cl, is not entirely unexpected. Counterintuitive compounds (such as LiH_2 , LiH_6 , and LiH_8) have been predicted (21) to appear under pressure, but experiments have so far failed to find them (22). Our results suggest that new stable compositions with unusual chemical bonding may exist in other simple systems, such as K-Cl, but also in important planet-forming systems such as Mg-Si-O (23) and H-C-N-O. Furthermore, these results point to possibilities for creating materials with unusual properties that may be quenchable to ambient conditions.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S12
Table S1
References (24–32)

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Brood Parasitism and the Evolution of Cooperative Breeding in Birds

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The global distribution of cooperatively breeding birds is highly uneven, with hotspots in Australasia and sub-Saharan Africa. The ecological drivers of this distribution remain enigmatic yet could yield insights into the evolution and persistence of cooperative breeding. We report that the global distributions of avian obligate brood parasites and cooperatively breeding passerines are tightly correlated and that the uneven phylogenetic distribution of cooperative breeding is associated with the uneven targeting of hosts by brood parasites. With a long-term field study, we show that brood parasites can acquire superior care for their young by targeting cooperative breeders. Conversely, host defenses against brood parasites are strengthened by helpers at the nest. Reciprocally selected interactions between brood parasites and cooperative breeders may therefore explain the close association between these two breeding systems.

Cooperative breeding, in which three or more individuals contribute to the care of young in the nest, occurs in around 9% of birds (1). The distribution of this social system is strongly skewed toward two major hotspots: Australasia and sub-Saharan Africa (2) (Fig. 1A). Ecological correlates of this distribution include both variable, unpredictable environmental conditions (2) and stable, predictable conditions (3). Unsurprisingly, the broad-scale ecological conditions that favor the evolution and persistence of cooperative breeding in birds therefore remain controversial (2, 4, 5).

Previous studies have proposed that cooperatively breeding species are more likely to be hosts of avian interspecific brood parasites than are noncooperative species (6, 7). We investigated the correlation between avian brood parasitism and cooperative breeding. Interspecific brood parasites lay their eggs in the nests of other birds, primarily passerines, and abandon their young to the care of the host (8). The cost of hosting a brood parasite can be immense, so hosts are typically under selection to evolve defenses against parasitism (8). One of the most ubiquitous host defenses is the mobbing of brood parasites (9). Collective mobbing by multiple individuals can provide a more effective defense than solitary or pair mobbing, thus providing a selective force for cooperative or colonial breeding (10).

To test this hypothesis, we first compared the global geographic breeding distribution of avian brood parasites and cooperatively breeding passerine species (11). We found a strong correlation between species richness in cooperative breeders and species richness in brood parasites [simultaneous autoregressive model (z) = 61.3, $P < 0.0001$, correlation coefficient (r^2) = 0.68, Fig. 1],

with both exhibiting the same geographic skew toward sub-Saharan Africa and Australasia [63% of avian brood parasite species breed exclusively within this region (8)]. This correlation remains strong after controlling for avian species richness ($z = 21.0$, $P < 0.0001$, $r^2 = 0.41$, fig. S1).

This correlation could reflect a direct association between brood parasitism and cooperative

breeding, or both breeding systems could be the outcome of a third variable, such as the high cost of parental care in variable environments (2). If there is a direct association, either because exploitation by brood parasites promotes cooperative breeding or because brood parasites favor cooperatively breeding hosts, we would predict that within a given geographic region, species that are hosts of brood parasites should be more likely to breed cooperatively than nonhosts. We tested this prediction using phylogenetic comparative methods for two regions with sufficiently well-studied avifaunas: Australia and southern Africa (11). These two regions encompass the phylogenetically diverse passerine and nonpasserine hosts of 21 cuckoo species, 6 honeyguide species, and 9 parasitic finch species. We used published classifications of the host status of Australian passerines [brood parasites exploit passerines exclusively in Australia (12)] and all southern African birds (13) and the modes of parental care in all bird species worldwide (1). Our analyses revealed a significant association between hosts of brood parasites and cooperative breeders in both southern Africa (Bayes factor = 18.36, strongly correlated; likelihood ratio test: $\chi^2 = 60.28$, $P < 0.001$; Fig. 2A) and Australia (Bayes factor = 17.34, strongly correlated; like-

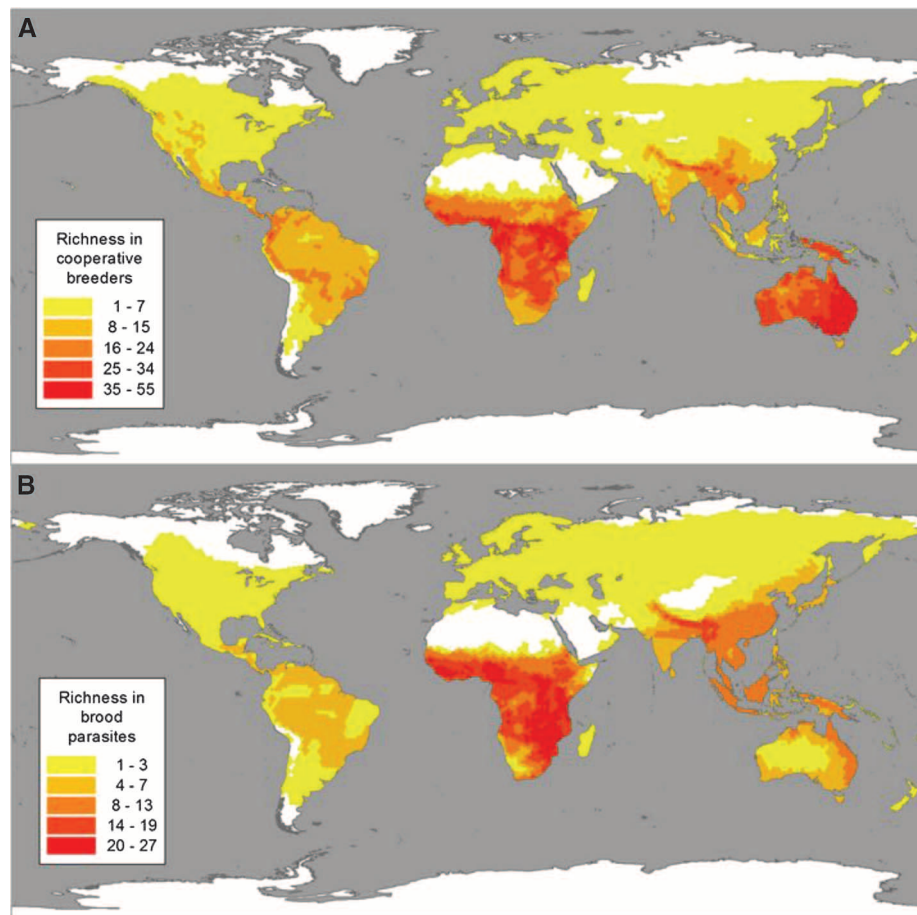


Fig. 1. Global patterns of richness in (A) avian cooperatively breeding passerine species and (B) obligate avian brood parasite species, during their breeding seasons.

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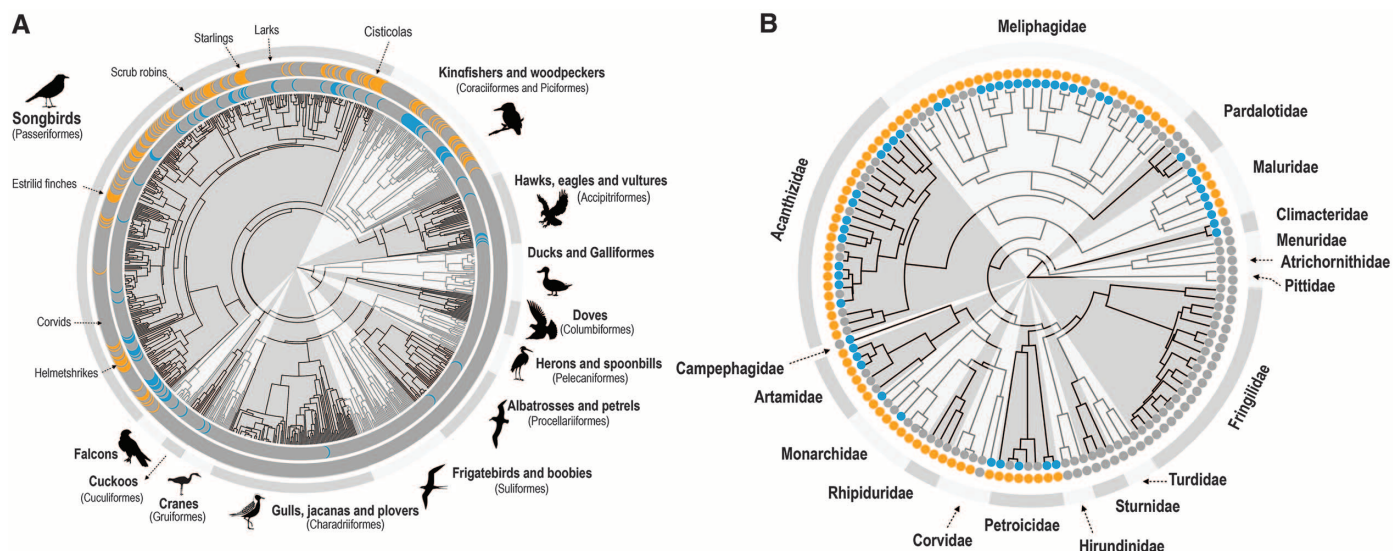


Fig. 2. Random phylogenetic trees for (A) 892 bird species in southern Africa (gray divisions represent orders) and (B) 129 passerine species in Australia (gray divisions represent families). Orange circles indicate cuckoo hosts, and blue circles indicate cooperative breeders.

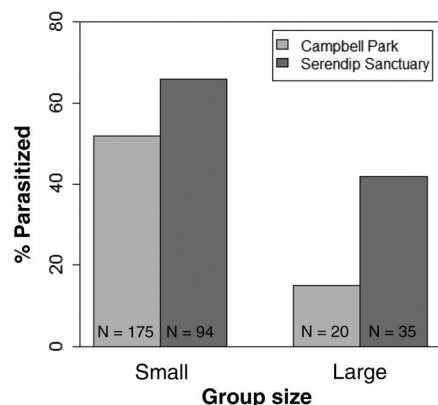


Fig. 3. The percentage of large and small superb fairy-wren groups that were parasitized by Horsfield's bronze cuckoos at Campbell Park and Serendip Sanctuary, Australia.

likelihood ratio test: $\chi^2 = 11.66$, $P = 0.02$; Fig. 2B). In southern Africa, 27.5% of hosts were cooperative breeders, compared to only 7.6% of nonhosts. Similarly, in Australia 52.8% of hosts were cooperative breeders, compared to 11.9% of nonhosts.

Three non-mutually exclusive processes could explain why brood parasite hosts are more likely to be cooperative breeders: (i) Brood parasites might selectively target cooperative breeders to maximize the care of their offspring (7); (ii) cooperative breeders may be more obvious targets as a result of the increased activity of helpers near the nest (6, 7); and (iii) cooperative breeders may be better able to defend their nests against brood parasitism (7), selecting for cooperative breeding in hosts. To investigate whether one or more of these processes underpin the patterns uncovered by our comparative analysis, we conducted field observations and experiments on the facultatively

cooperative superb fairy-wren *Malurus cyaneus*. In this species, some pairs breed unassisted, whereas others are assisted by up to six nonbreeding helpers. This allowed us to investigate how cooperative breeding might change the outcome of interactions with brood parasites. In southeastern Australia, superb fairy-wrens are the primary host of Horsfield's bronze cuckoo, *Chalcites basalus* (12), and can suffer high annual rates of brood parasitism (14).

We began by investigating whether cuckoos might gain a selective advantage by preferentially targeting cooperative breeders for parasitism, using superb fairy-wren breeding and parasitism data (11). Cuckoo chicks grew slightly faster when reared by groups of three or more ($n = 30$ cuckoo chicks, day of the nestling period $\times$ group size; $F_1 = 7.46$, $P = 0.009$), with a predicted mean ($\pm$ SE) mass on day 12 of 22.6 g (± 0.5 g) if reared by a pair and 23.4 g (± 0.5 g) if reared by a group. The chance of surviving to fledge was also greater for nestlings reared by larger groups, because predation rates decreased with increasing group size [generalized linear mixed model (GLMM): $\chi^2_1 = 4.31$, $P = 0.04$]. Although superb fairy-wrens commonly reject cuckoo chicks (14), the incidence of chick rejection was not correlated with group size ($n = 72$ cuckoo chicks, logistic regression, $\chi^2_1 = 0.6$, $P = 0.44$). Overall, then, our analyses provide support for hypothesis (i). We find that brood parasites can gain a fitness advantage for their offspring by associating with cooperative breeders, because they offer superior provisioning and a more effective defense against predators.

However, our analyses also show that this potential fitness advantage was seldom realized by Horsfield's bronze cuckoos parasitizing superb fairy-wrens, even when considering data from two sites over 500 km apart. Large groups were significantly less likely to be parasitized than small

groups at both Campbell Park (GLMM: $\chi^2_1 = 7.68$, $P = 0.006$) and Serendip Sanctuary ($\chi^2_1 = 5.01$, $P = 0.027$; Fig. 3). Therefore, our results do not support hypothesis (ii): Cuckoos were not drawn to parasitize cooperative breeders because they are more salient targets for exploitation. Instead, we find support for hypothesis (iii), because cooperative breeding facilitates defense against brood parasites. We quantified the fitness advantage associated with better defenses against parasitism in large groups using data from Campbell Park (11). Relative to small groups, the reduced probability of parasitism in large groups increases the production of young by 0.2 fledglings per group per season. Therefore, both parents and related helpers gain a fitness advantage from cooperative breeding when interacting with brood parasites.

Subsequent experimental analyses of behavior at the nest revealed how larger groups are able to escape parasitism more frequently than smaller groups. We found that superb fairy-wrens were more aggressive toward mounts of a cuckoo than of a nest predator (eastern brown snake, *Pseudonaja textilis*), a predator of adult birds (collared and Eurasian sparrowhawk, *Accipiter cirrocephalus* and *A. nisus*, respectively), a predator of both adults and nestlings (pied currawong, *Strepera graculina*), or a nonthreatening control (white-plumed honeyeater, *Lichenostomus penicillatus*; linear mixed effects model on number of mobbing calls: $\chi^2_4 = 53.95$, $P < 0.0001$; Fig. 4A). Further, cuckoo-targeted mobbing was elicited by a referential vocalization. Superb fairy-wrens produce whining alarm calls (15) that are structurally unlike any other calls in their repertoire (Wilk's $\lambda = 0.11$, exact $F_{8,56} = 13.75$, $P < 0.01$; fig. S3) and do so exclusively when confronting a cuckoo (Friedman test: $\chi^2_4 = 54.72$, $P < 0.0001$; Fig. 4C). With playback experiments, we found that this call elicits a more rapid approach by group members than mobbing alarm calls or a

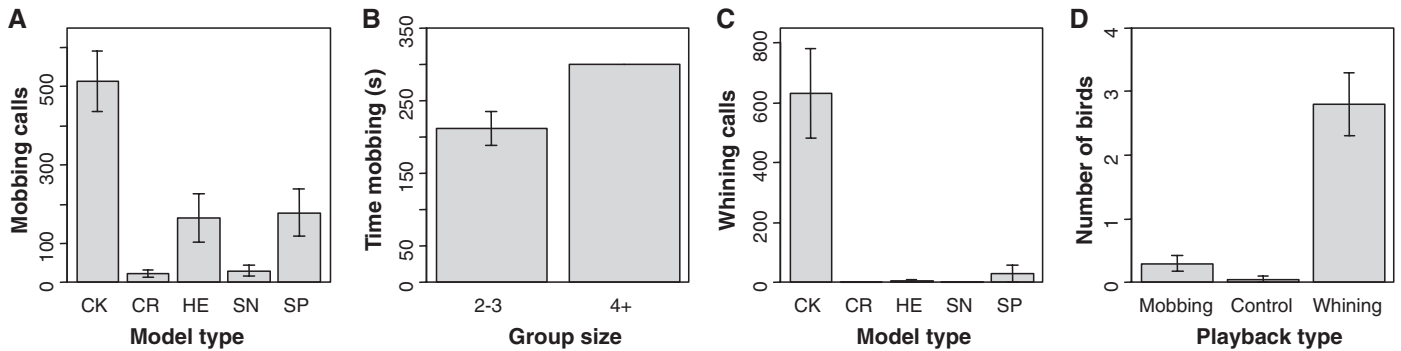


Fig. 4. (A) Mean number of mobbing alarm calls produced by 15 fairy-wren groups in response to different model types: cuckoo (CK), currawong (CR), honeyeater (HE), snake (SN), and sparrowhawk (SP). (B) Mean time spent mobbing the cuckoo model (<0.5 m from the model) by small ($n = 27$

groups) versus large ($n = 5$ groups) groups. (C) Mean number of whining calls produced in response to the five model types. (D) Mean number of individuals that approached playbacks ($n = 20$ each) of fairy-wren mobbing alarm calls, a control sound, and fairy-wren whining alarm calls. Error bars denote the standard error.

control sound (a parrot call, GLMM: $\chi^2_2 = 68.05$, $P < 0.0001$; Fig. 4D). Once mobilized, the strength of these defenses increases with group size. Large groups were more vigilant around their nest (GLMM: $\chi^2_1 = 8.03$, $P < 0.004$), spent more time mobbing the cuckoo than smaller groups (Kruskal Wallis test: $\chi^2_1 = 5.42$, $P = 0.02$; Fig. 4B), and ultimately were less likely to be parasitized. Thus, superb fairy-wrens possess cuckoo-specific nest defenses, which are enhanced by helper contributions and which can explain the lower parasitism rates experienced by large groups.

Our findings show a pronounced association between avian brood parasitism and cooperative breeding in birds, on a global scale. Our field data suggest that a two-way process underpins this relationship. On the one hand, brood parasites can gain a fitness advantage by preferentially exploiting the superior care provided by cooperatively breeding groups. On the other hand, the genetic relatives of offspring raised by cooperatively breeding families potentially gain fitness from the superior defenses that the extended family collectively mounts against brood parasites. Defense against brood parasitism is therefore an important kin-selected fitness advantage associated with cooperative breeding [see also (16)]. In superb fairy-wrens, we have shown this two-way process at work, but in other cooperatively breeding hosts, especially those with a less protracted coevolutionary relationship with brood parasites, only the first part of the process may be evident. The challenge remaining for future work is to determine the extent to which brood parasites have influenced the biology of cooperatively breeding species.

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Supplementary Materials

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C57BL/6N Mutation in *Cytoplasmic FMRP interacting protein 2* Regulates Cocaine Response

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The inbred mouse C57BL/6J is the reference strain for genome sequence and for most behavioral and physiological phenotypes. However, the International Knockout Mouse Consortium uses an embryonic stem cell line derived from a related C57BL/6N substrain. We found that C57BL/6N has a lower acute and sensitized response to cocaine and methamphetamine. We mapped a single causative locus and identified a nonsynonymous mutation of serine to phenylalanine (S968F) in *Cytoplasmic FMRP interacting protein 2* (*Cyfp2*) as the causative variant. The S968F mutation destabilizes CYFIP2, and deletion of the C57BL/6N mutant allele leads to acute and sensitized cocaine-response phenotypes. We propose that CYFIP2 is a key regulator of cocaine response in mammals and present a framework to use mouse substrains to identify previously unknown genes and alleles regulating behavior.

The reference mouse strain, C57BL/6J, was established in 1921 and has been maintained at the Jackson Laboratory since 1948 (1). In 1951, a colony of C57BL/6J was shipped to the National Institutes of Health (NIH),

and C57BL/6N became a second major source of C57BL/6 mice. Large-scale projects use different C57BL/6 substrains, including the International Knockout Mouse Consortium (IKMC), which uses C57BL/6N embryonic stem (ES) cells (2, 3), and

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the Mouse Genome Sequencing Consortium and Allen Brain Atlas, which use C57BL/6J (4, 5). Although most behavioral measurements to date have been carried out in the C57BL/6J substrain, the IKMC will shift emphasis to the C57BL/6N substrain. Although behavioral differences have been noted in C57BL/6 substrains, their genetic basis has not been elucidated (6, 7). Therefore, it becomes important to understand the genetic causes of phenotypic differences between C57BL/6N and C57BL/6J.

Drug addiction is characterized by loss of control over drug consumption and behaviors associated with drug seeking. Even though drugs of

abuse fall into different pharmacological categories, they all act on the mesolimbic reward pathway in the brain (8). Long-term structural and functional changes in neuronal circuitry are thought to be a key feature of addiction (9). We investigated in detail a difference in cocaine response between the C57BL/6N and the C57BL/6J mouse substrains.

We characterized C57BL/6N and C57BL/6J animals for locomotor response to cocaine. Because of the nonlinear nature of drug response, we carried out testing at multiple doses. C57BL/6N had a 45% (1 SD) lower acute response to cocaine and methamphetamine at multiple doses (Fig. 1, A

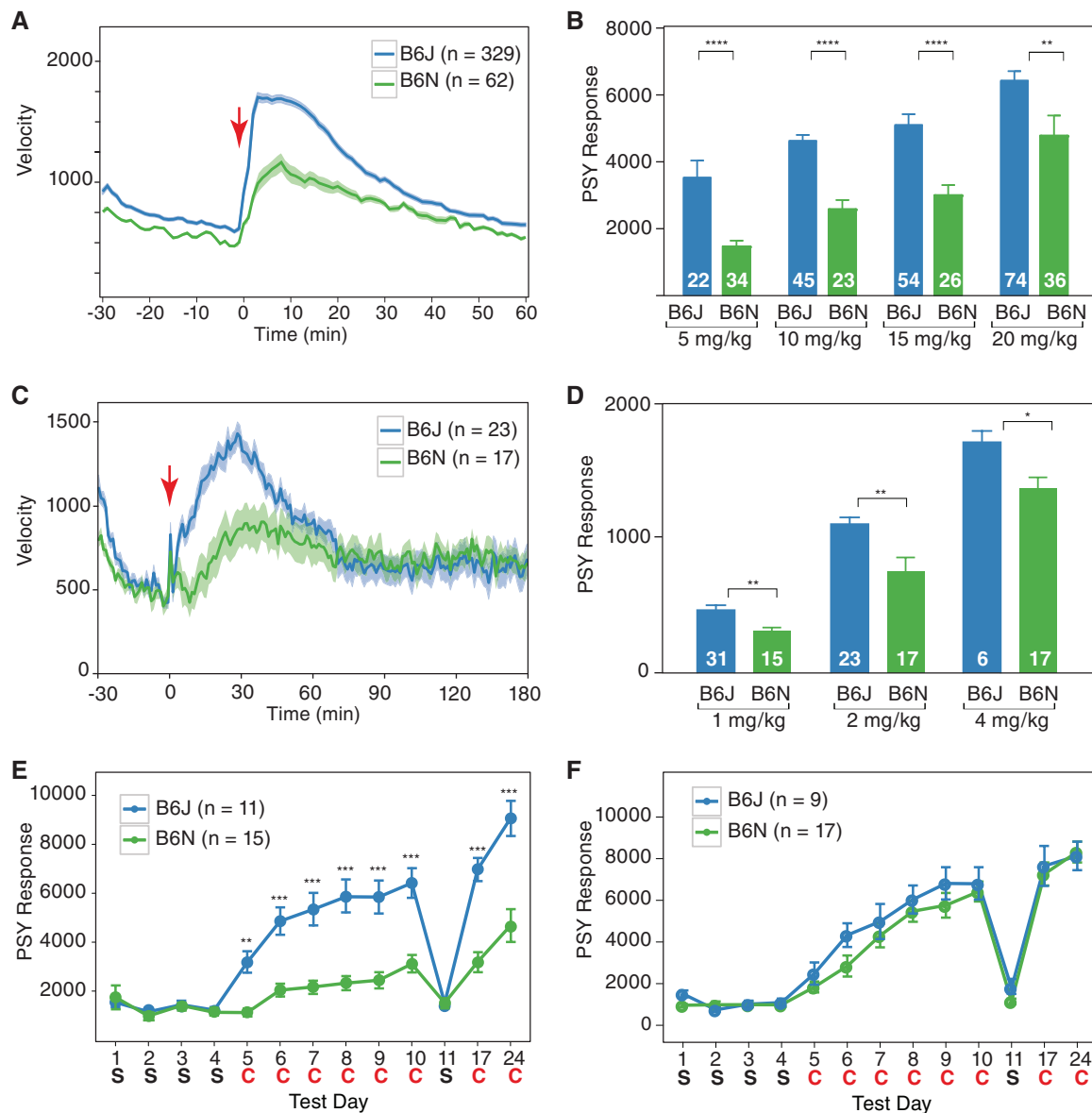


Fig. 1. Acute and sensitized psychostimulant response, measured by locomotor hyperactivity, is lower in C57BL/6N (B6N) substrain than in C57BL/6J (B6J). (A) Baseline locomotor velocity data of B6J (blue) and B6N (green) mice were measured for 30 min, and then mice were injected with cocaine (20 mg/kg, red arrow) and recorded for 60 min further. (B) Cocaine dose response for B6J and B6N. (C) Decreased methamphetamine response in B6N. Animals were injected with methamphetamine (2 mg/kg, red arrow) and recorded for 3 hours. (D) Methamphetamine dose response for B6J and B6N.

Mice were treated with 1, 2, and 4 mg/kg of the drug. Average response 60 min post injection is shown. (E and F) B6N have a lower sensitized response to cocaine at 10 mg/kg (E) but respond similarly at 15 mg/kg dose (F). Mice were measured for baseline and postinjection response for 1 hour and injected with saline (S) or cocaine (C). The 30-min postinjection response is shown. All data are shown as mean $\pm$ SEM; the number of animals in each group is indicated. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ from Tukey post hoc test following two-way analysis of variance (ANOVA) [(B) and (D)] or pairwise t test [(E) and (F)].

to D, and fig. S1). In psychomotor sensitization, repeated administration of the stimulus elicits a progressively larger behavioral response. It is regulated by experience-dependent neuronal plasticity and thought to be a key event leading to addiction (10, 11). At a 10-mg/kg dose, C57BL/6J sensitized to cocaine much more efficiently than C57BL/6N (compare day 5 to 10, Fig. 1E), although both strains sensitized to similar levels at a higher 15-mg/kg dose (Fig. 1F).

To determine which genetic loci contribute to reduced cocaine response seen in C57BL/6N, we carried out quantitative trait locus (QTL) analysis (fig. S2). Parental strains, F₁, and F₂ animals were tested concurrently. C57BL/6N had an ~1-SD lower response to cocaine. The F₁ animals exhibited a cocaine response similar to that of C57BL/6N, indicating dominance of the C57BL/6N allele, and the F₂ progeny were intermediate in response when compared with the parental strains (Fig. 2A). These differences were seen in all of our measures and in both sexes (figs. S3 and S4).

To perform QTL analysis, we identified polymorphic markers between C57BL/6J and C57BL/6N (supplementary materials, fig. S5, and tables S1 and S2). QTL analysis for cocaine response

yielded a single QTL on chromosome 11 [log of odds (LOD) = 6.8] (Fig. 2C). This peak is highly significant on the basis of permutation tests (Fig. 2C), is specific to cocaine response, and was not seen for baseline activity (fig. S6). The peak linkage occurs at marker rs13481014 for 30 min, 60 min, and net response (Fig. 2D), and the genotype effect plot indicates that the B6N/B6N allele elicits a lower response than the B6J/B6J allele. The F₁ response is similar to that of C57BL/6N, implying dominance of B6N allele (Fig. 2E). This QTL accounts for 11% of the total phenotypic variance (genetic, environmental, and error) and 61% of the genetic variance (fig. S7). The QTL support interval translates to a 22-Mb interval between 35 to 57 Mb of chromosome 11 (12) (supplementary materials and fig. S8).

Phenotypic differences between related strains may occur because of three possibilities: residual heterozygosity at the time of separation, genetic contamination after separation, or genetic drift. Genotyping indicated that inbreeding was essentially complete and that there was no genetic contamination or residual heterozygosity in C57BL/6N and C57BL/6J substrains (13). Because C57BL/6N and C57BL/6J diverged 62 years ago, there may be a limited number of polymorphisms accumu-

lated through genetic drift that accounts for the phenotypic difference. To identify the causative mutation, we performed whole-genome sequencing runs and combined the data with published and unpublished C57BL/6NJ sequences for 99.8-fold coverage of chromosome 11 (table S3) (14). We reanalyzed the data sets with identical parameters (fig. S9 and tables S4 and S5) and found a single nonsynonymous single-nucleotide polymorphism (SNP) in the QTL interval (Fig. 3A and fig. S10). No indels or structural variants cause protein-coding changes (Fig. 3, B and C). This nonsynonymous SNP changes G to A at 46,036,117 base pair (bp) of chromosome 11 (mm9) and produces a serine-to-phenylalanine missense mutation at position 968 (S968F) in *Cyfp2* (Fig. 3D). This variant had a high Polyphen2 score of 0.957/1 and was predicted to be damaging (15) (fig. S11). CYFIP1 and CYFIP2 are highly conserved proteins implicated in Fragile X-mental retardation protein (FMRP) function and regulation of actin polymerization through the WAVE regulatory complex (WRC) (16, 17). *Cyfp2* is widely expressed throughout the brain, whereas *Cyfp1* is more restricted (fig. S12). FMRP is the most common cause of mental retardation in humans, and WAVE complex members have been shown to regulate neu-

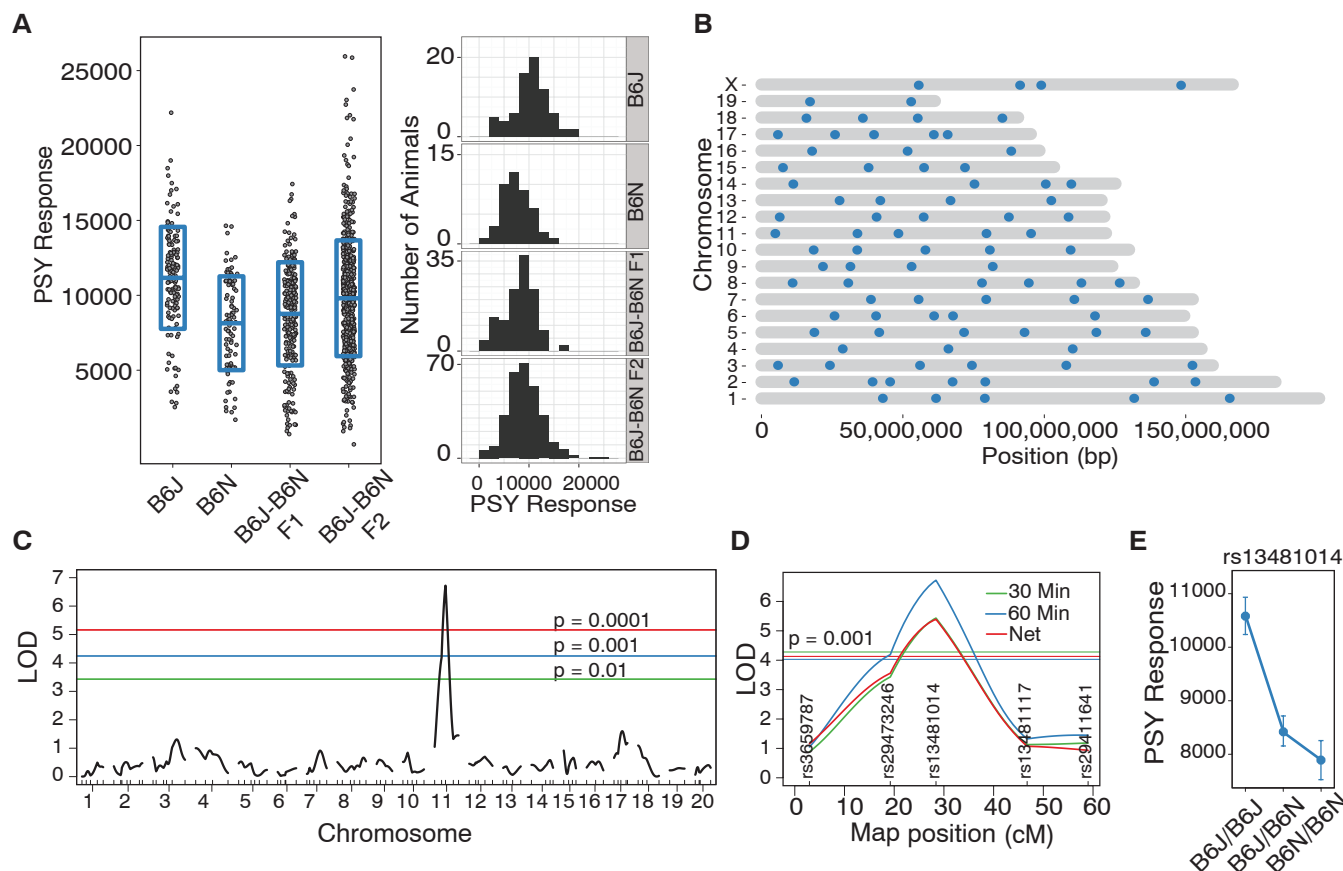


Fig. 2. QTL on chromosome 11 regulates cocaine response. (A) Hyperactivity after intraperitoneal injection of cocaine (20 mg/kg) was quantified. B6J ($n = 73$), B6N ($n = 124$), F1 ($n = 244$) were tested. The blue boxes represent mean $\pm$ 1 SD ranges, and the histograms show normal distributions of the measures. (B) Locations of polymorphic markers between B6J and B6N. (C) Genome-wide QTL scan revealed a

single highly significant QTL on chromosome 11. The significance thresholds are established through 100,000 permutation tests. (D) The chromosome 11 QTL is significant for multiple cocaine response measures. (E) Genotype effect plot at marker rs13481014 shows the B6N allele has lower response than B6J allele, and the F1 is intermediate. Data are mean $\pm$ SEM.

ronal connectivity and behavior in mice and *Drosophila* (18–23).

Because many C57BL/6 substrains are readily available with information about the time of separation from the founder colony, we constructed a phylogenetic timeline of the S969F variation (Fig. 3, E and F). The *Cyfp2* polymorphism was fixed in the C57BL/6N colony sometime between 1961 and 1974 and is present in most commercially available sources of C57BL/6N, including Charles River (NCr) and Taconic (NTac) (Fig. 3F).

The *Cyfp* gene family is highly conserved in metazoans, and a multiple-sequence alignment indicated that CYFIP2 S968 is 100% conserved in orthologs from *Caenorhabditis elegans* to hu-

mans (fig. S13A). The crystal structure of CYFIP1 has been solved as part of a pentameric 400-kD WRC (16). Molecular modeling of the S969F mutation using the WRC homology model revealed that the mutation leads to substantial steric hindrance with adjacent residues (figs. S13B and S14). We measured protein stability after cycloheximide treatment to estimate the relative half-life of wild-type and mutant CYFIP2. The wild-type and mutant proteins had half-lives of 8.5 and 2.8 hours, respectively, indicating that the S968F mutation greatly destabilizes CYFIP2 (Fig. 4A) (supplementary text). Stably transfected HEK293 cell lines expressing equivalent levels of the wild-type and mutant CYFIP2 proteins were used for biochemical and proteomic analyses. Quan-

titative co-immunoprecipitation (IP) assays showed no difference in interactions between wild-type and mutant CYFIP2 with NAP1, WAVE1, ABI2, and HSPC300, the core components of the WRC (Fig. 4B). We did not observe differences in our proteomic analysis (fig. S15) and saw no interaction of wild-type or mutant CYFIP2 with FMRP, FXR1, or FXR2 under our conditions.

To generate a second mutant allele of *Cyfp2*, we produced mice using ES cells carrying the “knockout first” (null) allele from the IKMC (3) and maintained them in C57BL/6N background (fig. S16). The homozygous knockouts die within a few hours of birth; however, the heterozygous mice are viable with no overt phenotypes. Because the ES cells used in IKMC are of C57BL/6N origin, the wild-type mice carry the mutant C57BL/6N alleles of *Cyfp2* (designated *Cyfp2*^{B6N/B6N}), and the heterozygotes carry one copy of the C57BL/6N allele (designated *Cyfp2*^{B6N/-}). We compared mice heterozygous for the knockout allele with the homozygous WT mice for cocaine response (Fig. 4C). As expected the C57BL/6N mice have lower cocaine response than C57BL/6J mice at all doses (Fig. 4C). The *Cyfp2*^{B6N/B6N} mice also have low response to cocaine, similar to that of C57BL/6N; however, heterozygous *Cyfp2*^{B6N/-} mice have higher cocaine response than homozygous *Cyfp2*^{B6N/B6N} mice at all three doses (Fig. 4C). The deletion of one mutant allele alleviates the severity of the phenotype seen with two mutant alleles and explains the dominant phenotype of the F1 progeny and the genotype effect plot of linked QTL maker seen previously (Fig. 2, A and E). The sensitized response is also higher in heterozygous mice (*Cyfp2*^{B6N/-}), confirming that CYFIP2 regulates acute and sensitized response to cocaine (Fig. 4D). We did not see any changes in cocaine metabolism between the two strains (fig. S17).

Next, we explored the functional effect of the CYFIP2 S968F mutation. Drug-induced structural plasticity is thought to be key in addiction. We measured and classified dendritic spine morphology of medium spiny neurons in the nucleus accumbens. C57BL/6N have a lower number of total spines with significantly lower spines of each class (Fig. 4E). Because dendritic spines are the major sites of excitatory postsynaptic current, we predicted a deficit in excitatory glutamatergic signaling in C57BL/6N. We measured the frequency and the amplitude of mini-excitatory postsynaptic signaling currents (mEPSCs) in the nucleus accumbens shell and found a decrease in frequency, but not amplitude, of mEPSCs (Fig. 4F). Lowered frequency of mEPSCs is a plausible functional consequence of reduced dendritic spines, although we cannot rule out other adaptations.

Our work has three major implications. First, we identify a mutation at the nucleotide level by using QTL analysis and provide evidence that CYFIP2 is a regulator of acute and sensitized cocaine response. Second, with over 20 commercially available C57BL/6 substrains, we describe

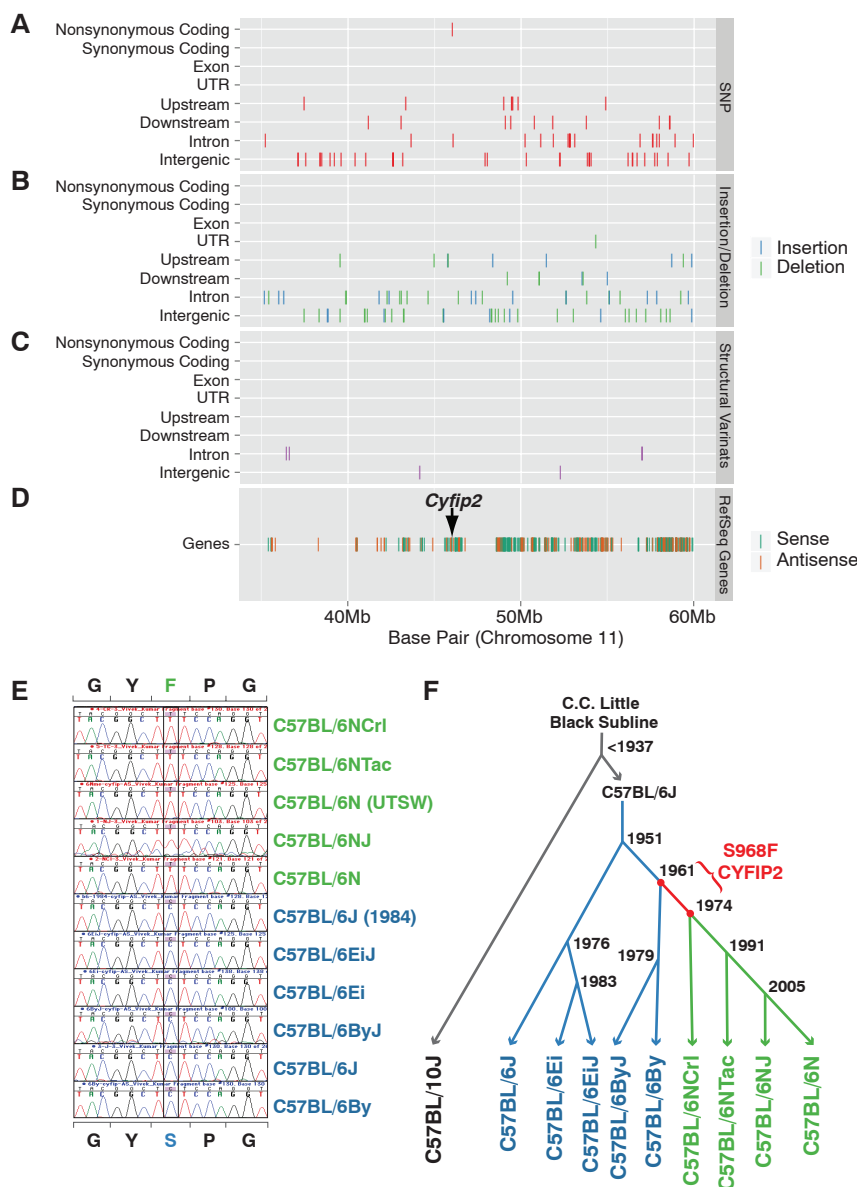


Fig. 3. Next-generation sequencing identifies a single nonsynonymous polymorphism in *Cyfp2*. Classification of SNPs (A), indels (B), and structural variants (C) sequencing reveals only a single SNP [top row of (A)] in *Cyfp2* (D) that changes Ser⁹⁶⁸ to phenylalanine in B6N. Only the QTL support interval is shown. UTR, untranslated region. (E and F) A comparison of C57BL/6 substrains shows that S968F variant arose in B6N lineage between 1961 and 1974.

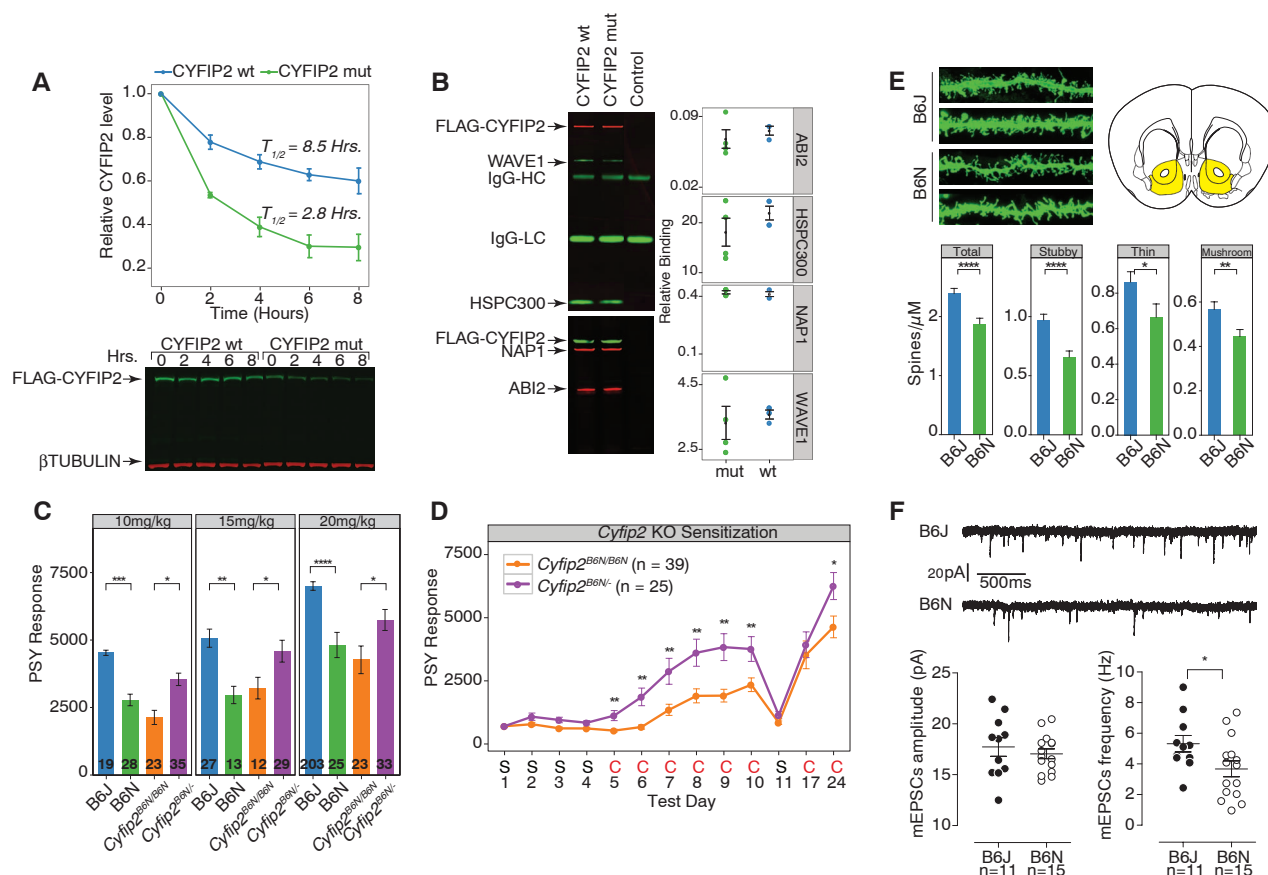


Fig. 4. CYFIP2 S968F causes destabilization, and knockout analyses of *Cyfip2* show that heterozygous mice have rescued cocaine response phenotypes. (A) Protein stability assay shows CYFIP2 S968F is less stable than wild type (WT). Human 293T cells transfected with FLAG-CYFIP2 (green bands) were treated cycloheximide for the indicated times, and Western blot was performed (bottom). β -tubulin was used as a control (red bands). Replicate experiments were quantitated, and half-life was calculated by using nonlinear one phase decay. K , the half-life parameter, is highly significant $P < 0.0001$ (top). (B) Quantitative IP experiments show CYFIP2 S968F interacts with WAVE complex members. IP (left) was conducted in replicate, and binding was quantitated (right). (C) Mice generated from ES cells harboring the knockout (KO) first allele of *Cyfip2* from the IKMC are on B6N background (designated *Cyfip2*^{B6N/B6N} for WT and *Cyfip2*^{B6N/-} for heterozygous knockout). The homozygous deletion is lethal at birth and therefore not shown. *Cyfip2*^{B6N/B6N} animals have lower cocaine response than the *Cyfip2*^{B6N/-} mice. (D) The *Cyfip2* heterozygous mice show higher sensitized response to cocaine than WT. *Cyfip2*^{B6N/B6N} (orange) and *Cyfip2*^{B6N/-} (purple) were injected with saline (S) or cocaine (C, 10 mg/kg). Pairwise comparisons for genotype for each day were significant as indicated. (E) Diolistic labeling of nucleus accumbens neurons shows decrease in dendritic spine density in B6N. Sample images are shown with representative location of neurons (yellow on coronal section of brain). Quantitation and classification of spines are shown. At least six animals were used in each group with over 10 images collected from each animal. (F) B6N has a decrease in frequency of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor mEPSCs. Sample traces of mEPSCs in nucleus accumbens shell medium spiny neurons from B6J and B6N mice. Dot plots for mEPSC amplitude and frequency. Data are mean $\pm$ SEM and Student's t test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Two-way ANOVA with Tukey post hoc test was used for (A).

a framework to use mouse substrains as a rich genetic source for identifying previously unknown genes and alleles that regulate behavior. If other substrains such as C3H/He and DBA/2 are included, there may be hundreds of mouse substrains available for analysis. Third, care must be taken when comparing new behavioral data from C57BL/6N substrains with existing data from C57BL/6J.

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Supplementary Materials

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Revealing Nature's Cellulase Diversity: The Digestion Mechanism of *Caldicellulosiruptor bescii* CelA

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Most fungi and bacteria degrade plant cell walls by secreting free, complementary enzymes that hydrolyze cellulose; however, some bacteria use large enzymatic assemblies called cellulosomes, which recruit complementary enzymes to protein scaffolds. The thermophilic bacterium *Caldicellulosiruptor bescii* uses an intermediate strategy, secreting many free cellulases that contain multiple catalytic domains. One of these, CelA, comprises a glycoside hydrolase family 9 and a family 48 catalytic domain, as well as three type III cellulose-binding modules. In the saccharification of a common cellulose standard, Avicel, CelA outperforms mixtures of commercially relevant exo- and endoglucanases. From transmission electron microscopy studies of cellulose after incubation with CelA, we report morphological features that suggest that CelA not only exploits the common surface ablation mechanism driven by general cellulase processivity, but also excavates extensive cavities into the surface of the substrate. These results suggest that nature's repertoire of cellulose digestion paradigms remain only partially discovered and understood.

In nature, most cellulolytic enzymes systems are of two general types: those with noncomplexed cellulases and hemicellulases produced by aerobic fungi and most bacteria (1), and those in which polysaccharidases self-assemble onto a common protein scaffold to form large macromolecular assemblies called cellulosomes (2, 3). Only a few anaerobic bacteria and fungi are known to produce cellulosomes. In both cases, the enzymes secreted are primarily equipped with a single catalytic domain. An alternate enzymatic system, in a sense midway between that of the two previous models, is used by the thermophile *Caldicellulosiruptor bescii* (previously *Anaerocellum thermophilum*) (4–6), and some other bacteria in which the individual cellulases secreted are multimodular, containing multiple binding and catalytic domains. The most abundant enzymes secreted by *C. bescii* are not only multimodular, but possess catalytic domains with different activities (multifunctional). Lochner and co-workers conducted an extensive characterization of the *C. bescii* secretome and concluded that CelA is the dominant cellulase (5). CelA is a complex, thermally stable enzyme containing an N-terminal family 9 glycoside hydrolase (GH9) endo- β -1,4-glucanase domain, three family 3 carbohydrate-binding modules (CBM3), and a C-terminal GH48 exo- β -1,4-glucanase domain. family 9 and 48 catalytic domains have been considered to be highly synergistic (7, 8). CelA was first isolated and partially characterized in terms of its activity by Zverlov and co-workers (9), who reported that CelA demonstrated activity on cellulose, as well as weak activity on xylan. However,

they did not report the performance of CelA on biomass and did not propose its mode of action or the complementarity of the two catalytic domains.

C. bescii can operate at temperatures up to 90°C (5, 6), which may explain the advantage

of several CBMs in counteracting the loss of binding due to increased temperature. Although *C. bescii* has been well studied at the microbial level, the structural features of its highly thermally stable enzymes have not been explained owing to the lack of structural information for enzymes from the *Caldicellulosiruptor* clade. Currently, large-scale biomass saccharification in bioconversion processes relies exclusively on fungal enzymes operating at 50° to 55°C. *C. bescii* CelA may have several advantages over other fungal and bacterial cellulases for use in biofuels production, namely, its high specific activity and stability at elevated temperatures.

Several recent studies report that the optimal growth temperature for *C. bescii* is near 80°C (5, 6). We examined the cellulolytic performance of purified CelA [supplementary material (SM) text S1 and S2] acting on Avicel, a model cellulose substrate that is generally used to evaluate cellulase action, at 60°, 75°, and 85°C. We compared CelA to a binary mixture containing *Trichoderma reesei* Cel7A exoglucanase, currently the most common exoglucanase found in commercial cellulase preparations (10, 11), and *Acidothermus cellulolyticus* Cel5A endoglucanase (11), which mimics the two cellulolytic (endo- and exoglucanase) activities found in CelA. The percentage of glucan converted over a 7-day digestion study is shown in Fig. 1A. CelA retained high activity compared

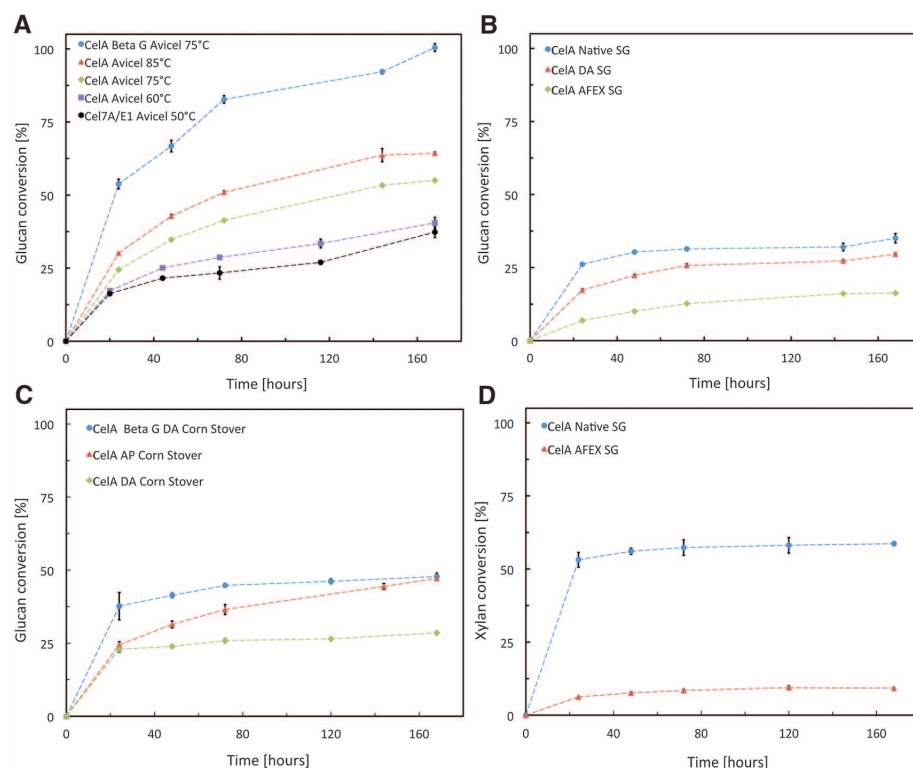


Fig. 1. Activity of CelA. (A) Avicel conversion by CelA at different temperatures (15 mg of CelA/g glucan or 14 mg of CelA + 1 mg β -glucosidase/g glucan) compared to Cel7A/E1 (12 mg/g + 3 mg/g glucan, respectively). (B) CelA conversion of switchgrass at 15 mg/g glucan. (C) Corn stover conversion by CelA at 20 mg/g (AP) or 15 mg/g (DA) glucan and improvement of CelA by addition of β -D-glucosidase on DA corn stover (14 mg CelA + 1 mg β -glucosidase/g glucan). (D) CelA conversion of xylan from native switchgrass at 15 mg/g glucan. Error bars represent standard deviations from three different experiments.

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to Cel7A at all temperatures tested, converting 60% of glucan at 85°C compared to 28% glucan conversion by Cel7A at its optimal temperature of 50°C (Fig. 1A). The extent of conversion obtained in this study for Cel7A is consistent with those reported by several other research groups (12–14). Furthermore, the activity of this enzyme acting on Avicel, on a molar basis, is seven times as high as that of the common exo- and endo-cellulase standard mixture, Cel7A and Cel5A (SM text S3). This high activity may be due to the proximity of a chain-end-forming endoglucanase and an efficient cellobiohydrolase in the same molecule, thereby increasing the intramolecular synergy. These results illustrate that when acting on Avicel, CelA is a far more active single enzyme than the dominant enzyme in today's commercial cellulase formulations, *T. reesei* Cel7A. End-product inhibition of cellulases is a well-known characteristic (15), and CelA in particular is known to produce both cellobiose and glucose. To assess this issue, we tested the sensitivity of CelA to cellobiose by adding low loadings of a thermally stable β -D-glucosidase from *Thermotoga maritima* (16) to the CelA mix. The addition of β -D-glucosidase markedly improved the performance of CelA acting on Avicel, producing 100% conversion in 7 days (Fig. 1A).

The performance of CelA acting on biomass is predictably lower than when acting on Avicel; CelA performs best on alkaline peroxide (AP)–(17, 18) pretreated corn stover (~50% conversion) (Fig. 1C). When tested on other substrates, such as dilute acid-pretreated switchgrass (DA) (19), native switchgrass (SG), and ammonia fiber expansion–treated switchgrass (AFEX) (20, 21) (Fig. 1B), CelA performed better on the untreated substrate even though the entire rationale of pretreatment is to improve enzymatic hydrolysis of biomass substrates. CelA activity was lowest when acting on AFEX-pretreated switchgrass, where the glucan conversion was 20%. Also, the activity of purified CelA on biomass was similar to that of the *C. bescii* purified secretome (ExtP) fraction (fig. S6). The importance of removing end-product inhibition when CelA acts on DA-pretreated corn stover is shown by the results in Fig. 1C, where CelA alone converts only ~30% of the glucan content to sugars and the addition of β -D-glucosidase increases this conversion by almost 75%: a notable improvement, given the relatively low overall enzyme loading.

While performing these digestion experiments, we also observed that CelA could achieve 60% conversion of xylan in native switchgrass, which showcases its potential for use in an industrial process using mild or no pretreatment (Fig. 1D). We tested both catalytic modules from CelA and found that the GH48 is largely responsible for this xylan-degrading activity (SM text S4). To further examine the xylan-degrading ability of CelA, we crystallized the two catalytic domains (GH9 and GH48) of CelA (SM text S5). It is probable that the additional ability to degrade xylans as well as glucans is defined by small

changes in the conformational properties of the active sites of glycoside hydrolases. However, closer examination of the CelA GH9 and GH48

structures shows no obvious features that would prevent or favor xylan binding over cellodextrins.

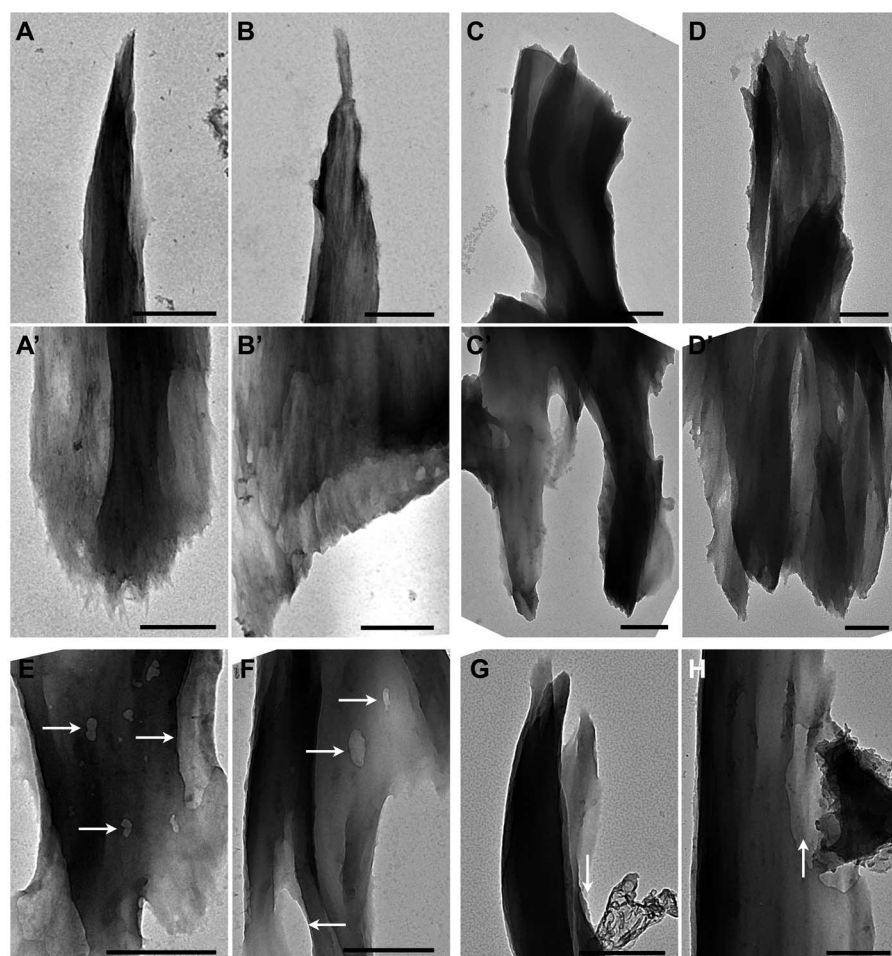


Fig. 2. TEM micrographs of partially digested small Avicel PH101 particles. Those digested to ~60% conversion by a CTec2 cellulase mixture (primarily comprising *T. reesei* Cel7A) display tip sharpening (A and B) on one end and blunted morphology on the other (A' and B'). Particles digested to ~65% conversion with CelA display slightly narrowed, tapered, blunt ends (C and D) and irregular blunt or angled ends (C' and D'), as well as cavities of various sizes on the surface [arrows, (E) and (F)]. Occasionally, some of the material being removed to form the cavities appears to remain attached to the cavity edge (G and H). All scale bars are 500 nm.

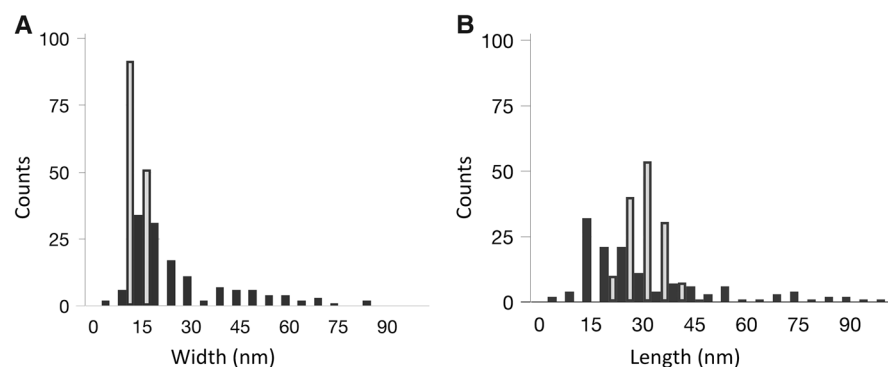


Fig. 3. Evidence that CelA fits in the cavities. (A and B) The histograms show dimensions of the cavities created by CelA (black), extracted from the TEM micrographs in Fig. 2, E and F, and the calculated dimensions of CelA itself (gray) over the course of a 40-ns molecular dynamics simulation, with the smallest dimension in (A) and largest dimension in (B).

Despite these compelling properties exhibited by CelA, several limitations may also be attributed to its multimodular architecture. Although the levels of glucan conversion achieved by CelA acting on highly crystalline cellulose (Avicel) are much higher than that of *T. reesei* Cel7A, the overall performance of CelA acting on commercially relevant feedstocks, such as switchgrass and corn stover, are lower, even with the use of a β -D-glucosidase (Fig. 1C). There are several important differences between biomass feedstocks and Avicel. Biomass is structurally and chemically more complex than Avicel. Smaller enzymes, such as Cel7A, may be better able to penetrate into the plant cell walls, even after pretreatment, whereas the larger CelA, with its multiple CBMs, may be too large and be more prone to nonproductive binding. Some of these characteristics may be enhanced by pretreatments that markedly change the structural integrity of the biomass, perhaps reflected by our observation that CelA works best with the AP-pretreated biomass, a pretreatment that substantially removes lignin (22, 23) (Fig. 1C).

Chemical species other than glucan present in biomass, such as lignin and hemicelluloses, may trap CelA in nonproductive binding states. Naturally, all cellulases will experience some level of nonproductive binding on biomass. However, owing to the larger molecular weight of CelA and because enzyme loadings are done on an enzyme weight basis, the molar enzyme loading of CelA is much lower than it is for Cel7A. Therefore, the activity of a CelA would be affected to a greater extent by nonproductive adsorption than would an equivalent mixture of cellulases with lower molecular

weights. This might explain the results on AFEX-pretreated biomass, where lignin-carbohydrate complexes (LCCs) are redeposited on plant cell walls after such pretreatment (22, 23). LCCs represent a barrier for all enzymes, but the problem seems amplified for a complex enzyme such as CelA.

Further comparison of CelA to its fungal counterparts reveals another fascinating aspect of this cellulase. Transmission electron microscopy (TEM) imaging results indicate that CelA has a mode of action distinct from that ascribed to fungal enzymes, such as those used in the commercial formulation CTec2 (Novozymes).

We examined changes in the surface and internal structure of Avicel PH101 particles treated with CelA. These particles were recovered from digestions carried out to ~65% cellulose conversion and then compared to Avicel particles digested to ~60% conversion using CTec2 (CTec2 is composed primarily of Cel7A). Our analysis focused on the most electron-translucent particles within each sample, where individual cellulose microfibrils could often be identified within the particles (Fig. 2). Particles digested by the Cel7A-containing formulation displayed morphology previously reported where one end of the particle was finely tapered to a narrow point (Fig. 2, A and B) and an opposite end displayed a blunt edge with a slight angle from the long axis (Fig. 2, A' and B') (24). CelA-digested particles, by contrast, displayed narrowed, irregular, but not finely tapered morphology on one end (Fig. 2, C and D) and an irregular, scalloped, angled morphology on the opposite end (Fig. 2, C' and D'). In addition to a surface ablation activity typical of fungal enzymes that seem to

work only on the surface of the substrate (Fig. 2, A and B), CelA appears to excavate down into the layers of the substrate, generating cavities (Fig. 2, E to H, and fig. S8). TEM evidence for this excavation mechanism was notable: CelA-digested Avicel particles displayed surfaces marked by irregularly spaced cavities distributed along the length of the particles (Fig. 2, E and F). The cavities vary widely in cross-sectional area at the surface between 25 and ~1000 nm². Most cavities are less than 500 nm² in cross-sectional area and have widths of 15 to 20 nm and lengths of 15 to 30 nm (Fig. 3). No cavities were observed on the surface of Avicel particles digested by CTec2. Additionally, on the basis of the predicted conformations that CelA can attain, as inferred by molecular dynamics simulations, it is clear that CelA fits into these cavities (Fig. 3). From these simulations, the effective size of CelA is estimated to be between 10 and 35 nm. These spatial dimensions for CelA correlate very well with the smaller cavity dimensions (diameter) in the range of 15 to 30 nm (Fig. 3). The schematic in Fig. 4 summarizes the differences in the digestion mechanism of CelA and Cel7A.

To better understand the digestion mechanism of CelA, we constructed a kinetic model of enzymatic digestion of cellulose to test the hypothesis that CelA has a specific mode of action that leads to cavity formation, a mode not found in simpler cellulase enzymes (SM text S6). In the model, CBMs are designed to bind and unbind from cellulose; catalytic domains have binding, engaging, and processive digestion functionality, with enhanced binding when a linker-attached module is bound. The model reproduces the surface ablation mode of digestion found in the commercial formulations when applied to enzyme with only a single CBM linked to a single catalytic domain (fig. S13C). The same digestion, dominated by single-layer ablation, is observed if identical functionality is used with the complex CelA-type model (fig. S10A). The model shows that the cavity formation occurs (fig. S10B) when the additional constraint is put into the complex model so that, once fully bound, the processive digestion is slowed owing to competing digestion directions and increased binding affinity, which inhibits repeated unbinding and rebinding in other locations.

On the basis of these results, it appears that CelA and multifunctional cellulases represent a distinct paradigm for cellulose digestion and high activity created by combining complementary modules separated by long linker peptides on the same gene product. We propose that this hydrolysis mechanism could synergize with other cellulases representing the previously known paradigms—free enzymes and cellulosomes—because they offer distinctive mechanisms. For example, in nature, enzymes from mesophilic saprophytic fungi are unable to mix with enzymes secreted into high-temperature environments. We thus propose that considerable synergism can be afforded if it is possible to bring together enzymes from these natural cellulolytic paradigms.

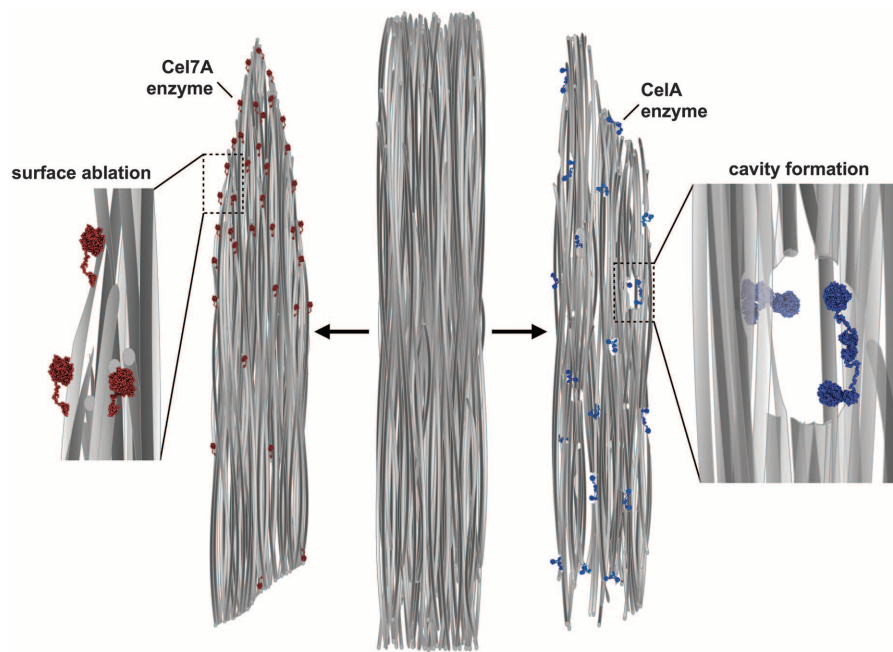


Fig. 4. Schematic representation of digested cellulose microfibril bundles. The diagrams contrast the surface ablation and reducing-end oriented mechanism of Cel7A (left) with the surface ablation and cavity-forming mechanism of CelA (right). This representation suggests how these distinct deconstruction mechanisms could be synergistic by specializing in different aspects of the nanoscale architecture of biomass materials and exposing new surfaces.

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Supplementary Materials

www.sciencemag.org/content/342/6165/1513/suppl/DC1

Materials and Methods

Figs. S1 to S17

Supplementary Text S1 to S6

Tables S1 to S8

References (25–35)

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Assembly and Validation of the Genome of the Nonmodel Basal Angiosperm *Amborella*

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Genome sequencing with next-generation sequence (NGS) technologies can now be applied to organisms pivotal to addressing fundamental biological questions, but with genomes previously considered intractable or too expensive to undertake. However, for species with large and complex genomes, extensive genetic and physical map resources have, until now, been required to direct the sequencing effort and sequence assembly. As these resources are unavailable for most species, assembling high-quality genome sequences from NGS data remains challenging. We describe a strategy that uses NGS, fluorescence in situ hybridization, and whole-genome mapping to assemble a high-quality genome sequence for *Amborella trichopoda*, a nonmodel species crucial to understanding flowering plant evolution. These methods are applicable to many other organisms with limited genomic resources.

Amborella (1, 2) has been identified as the single sister species to all other living angiosperms and is a pivotal reference for comparison to other angiosperms (3). However, *Amborella* is not a genetic model and has no existing genetic map, genetic resources, or genome sequence. Although next-generation sequencing (NGS) provides deep genomic sequence coverage at low cost, short-read assembly remains difficult, and assessing assembly accuracy is problematic without independently derived genomic maps. We produced a whole-genome assembly for *Amborella* from a mixed data set of 454, Illumina, and Sanger bacterial artificial chromosome (BAC)-end sequences, evaluated the assembly using fluorescence in situ hybridization (FISH), and improved contiguity using whole-genome mapping. FISH has broad utility (4), but has not been used in de novo genome assembly. Likewise, whole-genome mapping has been used

to assemble bacterial genomes (5, 6), but has only recently been applied to complex genomes of model organisms (7, 8) to assist with scaffolding and correction of well-advanced genome assemblies.

More than 23 Gb of quality-filtered (9) DNA sequence comprising single-end (SE) 454-FLX, SE 454-FLX+ reads, 11-kb paired-end (PE) 454-FLX, 3-kb PE Illumina HiSeq, and Sanger-sequenced BAC-end reads (10) were combined and assembled (table S1). Assembly (9) resulted in 5745 scaffolds totaling 706 Mb (table S5) with a mean scaffold size of 123 kb and an N50 size of 4.9 Mb, and N90 scaffold metrics that indicate that 90% of our assembled sequence resides within 155 scaffolds greater than 1.1 Mb in length (table S5).

Flow cytometry was used to estimate the size of the *Amborella* genome at ~870 Mb (11), while our sequence-based size assessments (9, 10, 12, 13) suggest that the *Amborella* genome size is closer

to 748 Mb. Our high-quality sequence represents an average depth of coverage of ~31×, and the assembly covers >94% of the genome.

Long contig and scaffold assemblies are required to understand genome structure, enable gene identification, and support subsequent comparative, structural, and population genomics studies. We sought long continuous stretches of assembled sequence that represent all, or a major fraction of, the *Amborella* genome. Coverage of two finished BAC contigs (10) by assembled sequence contigs suggests that these two regions were faithfully represented in the assembly (figs. S9 and S10) (9), and all 155 of our N90 scaffolds incorporate physically mapped BAC-end sequences.

The accuracy of the genome assembly was further assessed by FISH analysis (9). BACs assembled in 104 scaffolds containing 430 Mb (68%) of the genome assembly were cytogenetically localized by FISH to assess scaffold integrity (Fig. 1, fig. S11, and table S8). This analysis confirmed contiguity across major regions (56%) of 66 scaffolds containing 306 Mb (44%) of the genome assembly. Notably, co-assembled BACs that were

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cytogenetically mapped to different chromosomes indicated potential misassemblies in only two scaffolds (table S8). A karyotyping cocktail differentially labeled all 13 *Amborella* chromosome pairs and anchored major sections of 35 FISH-validated scaffolds to the karyotype (Fig. 2). In total, the cytogenetic cocktail directly placed 101 Mb (58%) of scaffolds with a total length of 176 Mb (~25%) of the assembly onto chromosomes (table S8). However, multiple BACs from 37 scaffolds containing

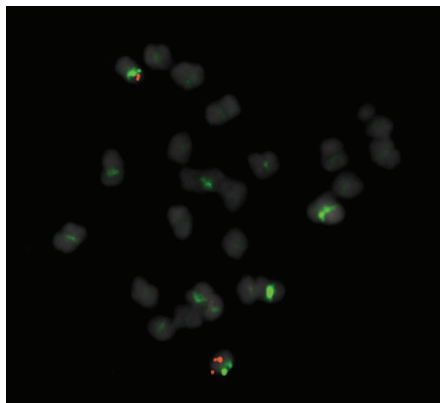


Fig. 1. FISH support of scaffold 7. Two BACs, AT_SB0003A05 (green) and AT_SB0003H23 (red), localize 8.2 Mb apart within the assembly scaffold 7 (9.5 Mb). Their colocalized FISH signals unambiguously support the assembly contained between their positional coordinates. Secondary green signals represent repetitive elements in AT_SB0003A05.

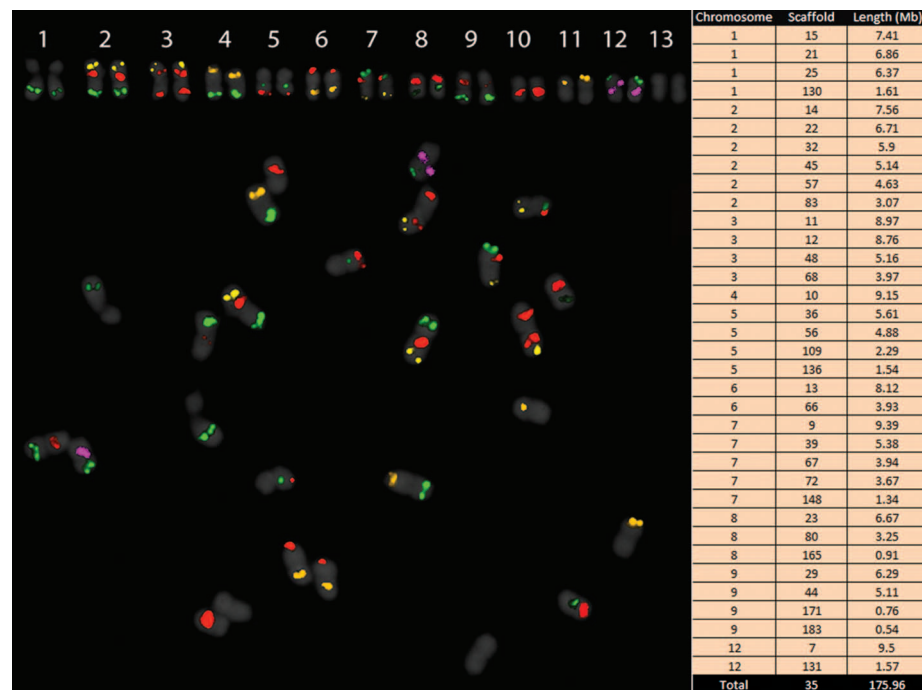


Fig. 2. FISH karyotype for *A. trichopoda*. BAC probes differentially label all chromosome pairs (one pair distinguished by the lack of fluorescent signal) and anchor 35 scaffolds (176 Mb) to the karyotype. Uniquely labeled chromosomes in the cytogenetic preparation (center) are arranged into homologous pairs (upper panel). Chromosomal assignments and sizes of cytogenetically localized scaffolds are tabulated.

154 Mb produced inconclusive genome-wide centromeric signals. Sequence alignments associated with the promiscuous probes indicate extensive sequence similarity and the presence of tandem repeats associated with the centromeric regions of the *Amborella* chromosomes.

Despite the extensive contiguity of the current draft assembly, gaps remain. Rather than constructing additional PE libraries to improve contiguity, a gap closure method based on whole-genome (formerly optical) mapping technology was undertaken in collaboration with OpGen, Inc. (Gaithersburg, MD, USA). Whole-genome mapping (14, 15) permits assembly of whole-genome restriction endonuclease maps by digesting immobilized DNA molecules and determining the size and order of fragments.

We compared assembled scaffold sequences to single-molecule restriction maps generated with *Amborella* genomic fragments to identify potential joins and produce superscaffolds (9) (table S10). This improved our original assembly by a 2× increase in both N50 (4.9 to 9.3 Mb) and N90 (1.2 to 2.9 Mb) (table S5). Thirty joins were confirmed through a new assembly constructed after adding an additional 454 PE sequences and improving data filtering, and 20 joins were confirmed by FISH (9) (table S10).

The *Amborella* assembly, as well as several recent plant whole-genome draft sequences (13, 16, 17), benefited from available collections of BAC-end sequences (10) that serve as very long (>150 kb) PE libraries. However, BAC clone

resources are expensive and time-consuming to construct and evaluate, as is end-sequencing by low-throughput and high-cost Sanger sequencing. Therefore, as improvement in NGS technologies enables more nonmodel eukaryote whole-genome sequence projects, it is important to identify methods that permit long, accurate assemblies in the absence of large-insert clone resources. Superscaffolding facilitated by Genome-Builder can substitute for BAC-end sequences, as illustrated by our construction of an *Amborella* assembly (9) (tables S11 to S13). Although BACs were used as FISH probes in this study, they are not required for cytogenetic validation of an assembly; alternatively, probes could be developed using polymerase chain reaction amplification. Thus, sequencing is no longer a limiting factor, and the greatest challenge for many organisms will be accurate and highly contiguous genome assembly. A combination of FISH and whole-genome mapping, in concert with sequence filtering and assembly strategies described here, should prove successful even for genomes with a more complex repeat structure than that of *Amborella*.

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Supplementary Materials

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CRL4 Complex Regulates Mammalian Oocyte Survival and Reprogramming by Activation of TET Proteins

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The duration of a woman's reproductive period is determined by the size and persistence of a dormant oocyte pool. Specific oocyte genes are essential for follicle maintenance and female fertility. The mechanisms that regulate the expression of these genes are poorly understood. We found that a cullin-ring finger ligase-4 (CRL4) complex was crucial in this process. Oocyte-specific deletion of the CRL4 linker protein DDB1 or its substrate adaptor VPRBP (also known as DCAF1) caused rapid oocyte loss, premature ovarian insufficiency, and silencing of fertility maintaining genes. CRL4^{VPRBP} activates the TET methylcytosine dioxygenases, which are involved in female germ cell development and zygote genome reprogramming. Hence, CRL4^{VPRBP} ubiquitin ligase is a guardian of female reproductive life in germ cells and a maternal reprogramming factor after fertilization.

In the mammalian ovary, primordial follicles are generated early in life and form a reservoir of female germ cells. To ensure a sufficiently long reproductive period, some primordial follicles must survive in a resting state for months (mice) or decades (humans) (1). However, the molecular mechanisms that control the balance between primordial follicle survival and loss are not well known.

The cullin family proteins assemble as many as 400 cullin-ring finger ligase (CRL) complexes that regulate diverse cellular pathways, but none have been functionally analyzed in oocytes. Cullin 4 (CUL4) utilizes damaged DNA binding protein-1 (DDB1) as a linker to interact with a subset of DDB1-cullin-associated factors (DCAFs), which act as substrate receptors (2, 3). Viral protein R (VPR)-binding protein, VPRBP (DCAF1), was an important CRL4 adaptor in these processes.

The CRL4 components CUL4A/B, ring of cullin-1 (ROC1), and DDB1 were highly expressed in mouse oocytes (figs. S1 and S2). We generated oocyte-specific and developmental stage-specific *Ddb1* knockout mice by crossing *Ddb1*^{fl/fl} mice with *Ddx4-Cre*, *Gdf9-Cre*, and *Zp3-Cre* transgenic mice (fig. S3A). For all resulting mouse strains, females were infertile (fig. S3B). In *Ddb1*^{fl/fl}; *Ddx4-Cre* mice, DDB1 was deleted in oocytes on postnatal days 1 to 3 (PDs 1 to 3) (fig. S3C). *Ddb1*^{fl/fl}; *Ddx4-Cre* ovaries at PD1 contained oocytes at numbers comparable to those in wild-type (WT) controls, whereas oocyte loss and apoptosis were notable at PD3 (Fig. 1, A to C, and fig. S3, D and E). All oocytes were lost in these mice by young adulthood (fig. S3F).

The ovaries of 6-week-old *Ddb1*^{fl/fl}; *Gdf9-Cre* females showed no histological abnormalities (fig.

S4, A and B). However, for *Ddb1*^{fl/fl}; *Gdf9-Cre* females older than 8 weeks, the ovaries were smaller than controls' (fig. S4A). Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) for MVH (oocyte marker) and FOXO1 (ovarian granulosa cell marker), respectively, indicated that oocytes and follicles were absent in *Ddb1*^{fl/fl}; *Gdf9-Cre* ovaries (Fig. 1D and fig. S4, C to E). Complete primordial follicle loss and gonadotropin level increases were observed in these mice within 12 weeks after birth and showed the premature ovarian insufficiency (POI) phenotype (fig. S4, B and F). However, for *Ddb1*^{fl/fl}; *Zp3-Cre* mice, DDB1 was intact in oocytes at the primordial follicle stage and was only deleted in activated oocytes. Abundant oocytes remained in *Ddb1*^{fl/fl};

Zp3-Cre ovaries at 12 weeks (Fig. 1D) to 8 months (fig. S4G) after birth. Thus, DDB1 was essential for oocyte maintenance at the primordial follicle stage.

VPRBP (DCAF1), one of the reported CRL4 adaptors, was also highly expressed in mouse oocytes (figs. S1 and S2). Therefore, we generated oocyte-specific *Vprbp* knockout mice (*Vprbp*^{fl/fl}; *Gdf9-Cre*). VPRBP was deleted in oocytes as early as PD10 (fig. S5A). *Vprbp*^{fl/fl}; *Gdf9-Cre* female mice were also infertile (fig. S3B). The ovaries of 12-week-old *Vprbp*^{fl/fl}; *Gdf9-Cre* mice were smaller than controls and lost all oocytes (Fig. 1D and fig. S5, B to E). Primordial follicles in *Vprbp*^{fl/fl}; *Gdf9-Cre* ovaries were depleted during PD12 to 14 (fig. S5, F and G). Apoptotic oocytes were frequently observed in PD13 *Vprbp*^{fl/fl}; *Gdf9-Cre* ovaries (fig. S6, A and B). Oocyte-specific *Ddb1* and *Vprbp* knockout mice were phenocopies of each other, which suggested that these proteins act in the same CRL4 complex to control primordial follicle development.

We postulated that the expression of oocyte-specific genes essential for primordial follicle survival might be altered by *Ddb1/Vprbp* deletion. Therefore, we determined the mRNA levels of oocyte-enriched genes in *Ddb1*^{fl/fl}; *Ddx4-Cre* ovaries at PD1. At this time point, the DDB1 protein had already been deleted in oocytes, although normal numbers of oocytes were still found in these ovaries (Fig. 1B and fig. S3C). Many genes essential for oocyte survival, were down-regulated in *Ddb1*^{fl/fl}; *Ddx4-Cre* ovaries, including *Sohlh1/2* (4, 5), *Nobox* (6), *Figla* (7), and *Kit* (8) (Fig. 2A). Decreased expression of essential oocyte genes was also observed in isolated DDB1-deleted oocytes (fig. S4H), as well as in *Vprbp*^{fl/fl}; *Gdf9-Cre* ovaries at PD12 (fig. S6C), when VPRBP has

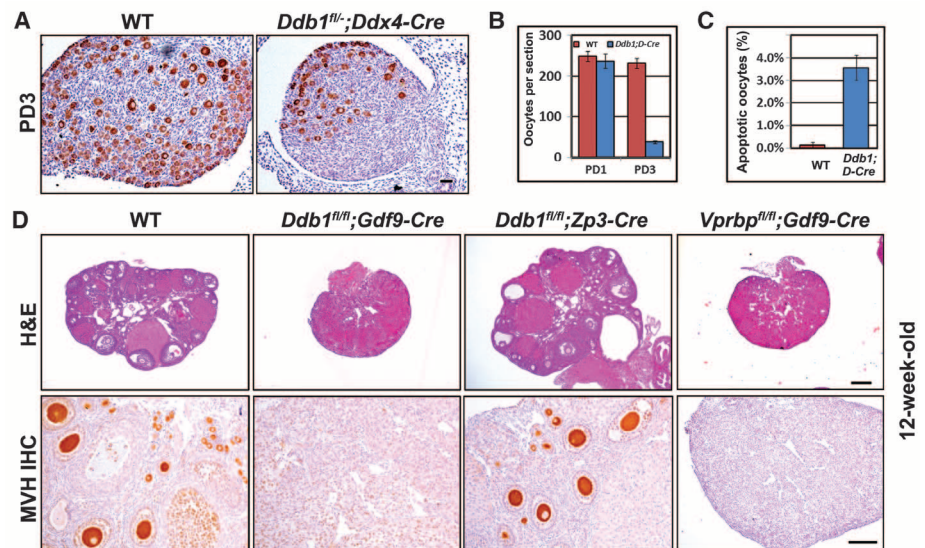


Fig. 1. CRL4^{VPRBP} in oocyte is required for primordial follicle maintenance. (A) MVH IHC staining in WT and *Ddb1*^{fl/fl}; *Ddx4-Cre* ovaries. Scale bars, 30 μ m. (B) Oocyte numbers in WT and *Ddb1*^{fl/fl}; *Ddx4-Cre* (*Ddb1*; *D-Cre*) ovaries at PDs 1 to 3. Error bars indicate SEM. (C) Quantification of apoptotic oocytes in WT and *Ddb1*^{fl/fl}; *Ddx4-Cre* ovaries in fig. S3E. (D) H&E staining and MVH IHC of ovaries from 12-week-old mice with the indicated genotypes. Scale bar, 250 μ m.

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been deleted in the oocytes but the primordial follicle numbers have not decreased (fig. S5G). In contrast, the mRNA levels of these genes were comparable to those in control ovaries at PD8 (fig. S6C), a time point before VPRBP deletion in oocytes (fig. S5A). These results indicated that

DDB1 or VPRBP deletion resulted in decreased expression of essential genes in oocytes, which contributed to the POI phenotype.

We then investigated the mechanism(s) that caused oocyte gene silencing after DDB1 or VPRBP deletion. Because a large numbers of genes were

down-regulated in DDB1- or VPRBP-deleted oocytes, we postulated that epigenetic changes in oocytes might have been involved. Indeed, the methylated CpG sites within *Gdf9*, *Figla*, *Nobox*, and *Ddx4* promoters were increased in *Ddb1^{fl/fl}*; *Gdf9-Cre* oocytes (Fig. 2B and fig. S7A). The paternal imprinting region, *H19* DMR, which was slightly methylated in control oocytes, exhibited increased CpG methylation in *Ddb1^{fl/fl}*; *Gdf9-Cre* oocytes (fig. S7A). These results indicated that decreased gene expression in *Ddb1^{fl/fl}*; *Gdf9-Cre* oocytes might be caused by dysregulated DNA methylation.

To elucidate the mechanism underlying the alteration of gene expression and methylation in *Ddb1*- or *Vprbp*-deficient oocytes, we considered the role of TET family DNA dioxygenases, which convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) (9, 10). Notably, 5hmC amounts in DDB1- or VPRBP-deleted oocytes were significantly lower than those in control oocytes at the primordial follicle stage (Fig. 2, C and D, and fig. S7, B and C). TET1, 2, and 3 were highly expressed in oocytes and early embryos (fig. S8A). The decrease in 5hmC generation in DDB1- or VPRBP-deleted oocytes suggested that CRL4^{VPRBP} might regulate TET activities.

Further evidence for the functional relevance of CRL4^{VPRBP}-TET interactions was apparent on the basis of the phenotypes of *Ddb1^{fl/fl}*; *Zp3-Cre* female mice. They did not have the POI phenotype but were infertile. Most *Ddb1^{fl/fl}*; *Zp3-Cre* oocytes failed to develop beyond the four-cell stage after fertilization (Fig. 3A and fig. S8, B and C). The expression of genes required for early embryo development, including *Nanog*, *Oct4*, and *Sox2*, were not induced in early embryos derived from DDB1-deleted oocytes (fig. S8D). Some other genes, such as *Yap* and the

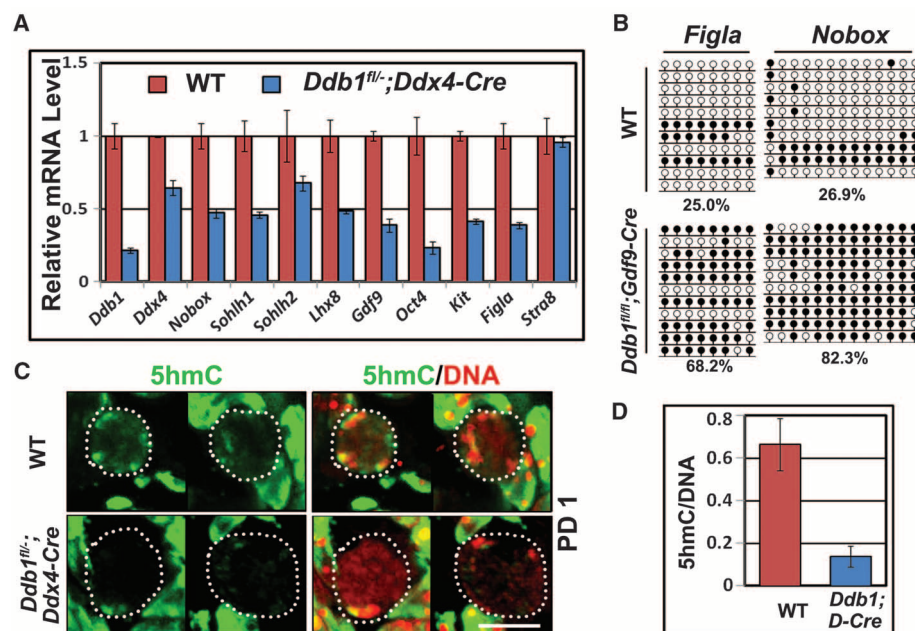


Fig. 2. CRL4^{VPRBP} is required for 5hmC generation and gene expression in primordial follicles. (A) Quantitative reverse transcription polymerase chain reaction results for the expression of the indicated genes in WT and *Ddb1^{fl/fl}*; *Ddx4-Cre* ovaries at PD1. The values of different genes in WT were set as "1." Error bars indicate SEM. (B) Increased DNA methylation in promoters of indicated genes in WT and *Ddb1^{fl/fl}*; *Gdf9-Cre* oocytes. Open and filled circles represent unmethylated and methylated CpGs, respectively. Percentages of methylated CpGs are indicated. (C) Immunofluorescence on ovarian sections showing 5hmC levels in primordial follicle stage oocytes (circled by white dots) of WT and *Ddb1^{fl/fl}*; *Ddx4-Cre* ovaries at PD1. Scale bar, 10 μ m. (D) Quantification of oocyte 5hmC signals shown in (C).

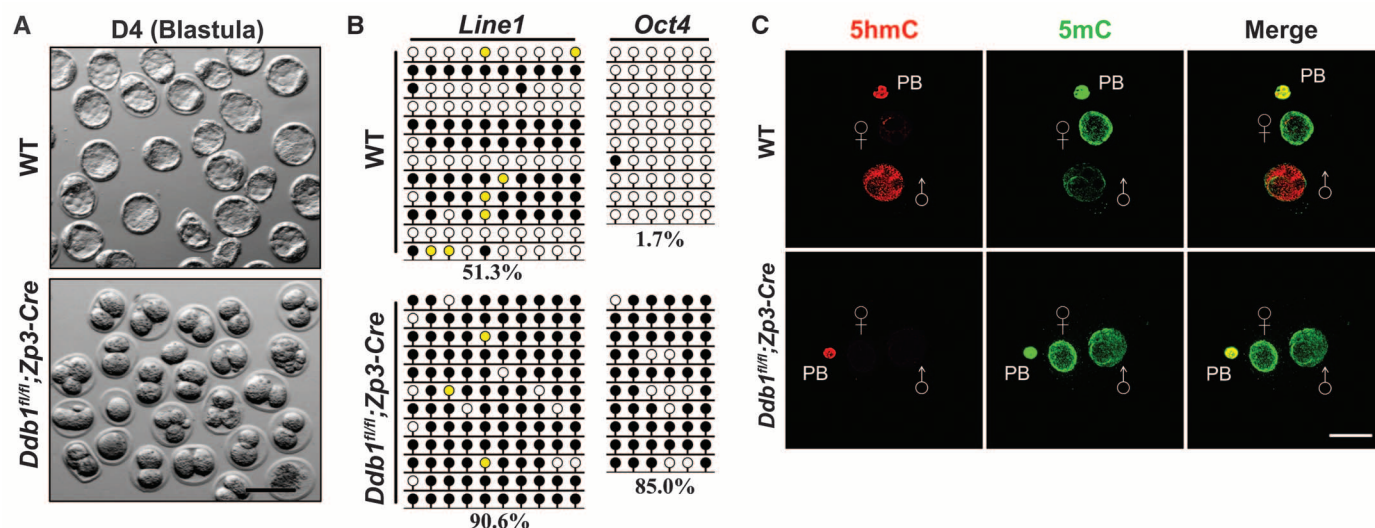


Fig. 3. CRL4^{VPRBP} is required for TET3-mediated 5hmC generation and zygotic genome activation after fertilization. (A) Morphology of blastulae derived from *Ddb1^{fl/fl}*; *Zp3-Cre* females mated with WT males. Scale bar, 100 μ m. (B) Methylation analysis of *Line1* and *Oct4* promoters in WT and *Ddb1^{fl/fl}*; *Zp3-Cre*

zygotes. Yellow circles represent unmatched CpGs. Percentages of methylated CpGs are indicated. (C) 5hmC/5mC immunostaining of zygotes from WT and *Ddb1^{fl/fl}*; *Zp3-Cre* females 24 hours after mating. PB, polar body. Female and male symbols indicate female and male pronuclei, respectively. Scale bar, 20 μ m.

CRL4 components *Cul4b* and *Roc1*, were unchanged. Note that *Tet1*, 2, and 3 mRNAs were increased in mutant embryos, possibly because of feedback of insufficient TET activity (fig. S8D). Demethylation of markers for successful paternal DNA reprogramming (*Line1* and *Oct4*) was also impaired in DDB1-deleted oocytes (Fig. 3B). These results suggested that DDB1 was a maternal factor required for zygotic genome activation.

TET3 is involved in epigenetic reprogramming of zygote paternal DNA after natural fertilization and in somatic cell nuclear reprogramming during animal cloning (11). CUL4B, DDB1, and VPRBP were all accumulated in pronuclei after fertilization (fig. S2). 5hmC and TET3 levels were high in the male pronuclei of WT zygotes, but were significantly decreased in zygotes derived from DDB1-deleted oocytes (Fig. 3C and fig. S9A). In somatic cell nuclear transfer experiments, somatic pseudopronuclei (PPN) underwent the 5mC-5hmC transition only in reconstructed zygotes derived from WT oocytes but not in those from *Ddb1*-null oocytes (fig. S9B). TET3 that originated from WT oocytes, but not from DDB1-deleted oocytes, became concentrated in those PPNs (fig. S9C). Thus, the absence of maternal DDB1 blocked zygotic genome reprogramming.

CRL ubiquitin ligase activation requires cullin neddylation, which is catalyzed by NEDD8-activating enzyme E1 (NAE1). To investigate whether CRL4 activity, in addition to the presence of DDB1 and/or VPRBP, was required for 5hmC generation in zygotes, we cultured WT zygotes with MLN4924, an NAE1 inhibitor. MLN4924 abolished cullin neddylation in oocytes (fig. S9D) and 5hmC generation in male pronuclei (fig. S9E). These results indicated that CRL4^{VPRBP} activity was required for TET-mediated 5hmC generation in fertilized oocytes or those with a transferred somatic nucleus.

To determine whether these were general effects, we used HeLa cells as a somatic cell model. DDB1 or VPRBP overexpression increased endogenous 5hmC levels. However, a mutated VPRBP (VPRBP-2RA) that could not bind to DDB1 failed to do so (Fig. 4A and fig. S10A). Overexpression of the TET1 catalytic domain (TET1-CD) induced a significant increase in 5hmC levels. However, this was abolished by DDB1 or VPRBP depletion or with MLN4924 treatment, although TET1 expression and nuclear localization were not affected (Fig. 4B and fig. S10B). By comparison, RNAi depletion of CRL4^{VPRBP} in HeLa cells (fig. S11), as well as MLN4924 treatment, markedly decreased their endogenous 5hmC levels (fig. S10, C to E). These results indicated that CRL4^{VPRBP} was required for TET-mediated 5hmC generation in both oocytes and somatic cells.

We next examined if CRL4^{VPRBP} physically interacted with TETs. Coimmunoprecipitation results indicated that TET1, 2, and 3 bound to VPRBP through their C-terminal CD (Fig. 4C and fig. S10F). VPRBP-WT bound to both DDB1 and TET3-CD (fig. S10, G and H). VPRBP-2RA

did not bind to DDB1 but still interacted with TET3-CD. Furthermore, the VPRBP N-terminal fragment bound to TET3-CD, although it did not contain the WD40 domain that is essential for DDB1-binding. These results indicated that VPRBP bound to the TET1/2/3-CD through its N-terminal region and bound to DDB1 through its WD40 domain close to its C terminus.

Finally, we investigated the role of CRL4^{VPRBP} in regulating TET1, 2, and 3 activities. TET1/2/3-CD purified from HeLa cells bound with DNA fragments amplified from *Gdf9* and *Fgf8* promoters or synthesized DNA probes. However, the TETs-DNA interaction was abolished by MLN4924 treatment (Fig. 4D and fig. S10I). Taken together, we established that CRL4^{VPRBP} was essential for TET1, 2, and 3 activities by promoting their DNA binding ability.

In recent years, phosphatidylinositol 3-kinase signaling in oocytes has attracted attention as a determinant of ovarian aging (12–14). However, whether other pathways in oocytes also have direct effects on maintaining the primordial follicle pool remains uncertain. Our results identified the

E3 ligase CRL4^{VPRBP} as a crucial factor for oocyte survival. Rather than activating primordial follicles, CRL4^{VPRBP} regulates their survival or loss to determine reproductive aging and menopause in females. The mouse models generated in this study (summarized in table S1) mimicked human POI patients better than previously reported *Foxo3a*- or *Pten*-deleted mice in several respects. For example, *Foxo3a* or *Pten* deletion in mouse oocytes resulted in (i) global activation of an ovarian primordial follicle pool; (ii) increased ovulation of activated oocytes; and, ultimately, (iii) female germ cell exhaustion (12, 14). However, the first two steps are not observed in most human POI patients. Typically, even if oocytes are eventually obtained from these patients, they fail to develop into healthy embryos after in vitro fertilization. These phenomena cannot be simply explained by rapid oocyte exhaustion. Our study with oocyte-specific CRL4^{VPRBP}-deficient mice provides new evidence that in genetically defective females that are destined for POI, mutated but otherwise “healthy-looking” oocytes could be epigenetically abnormal and have limited

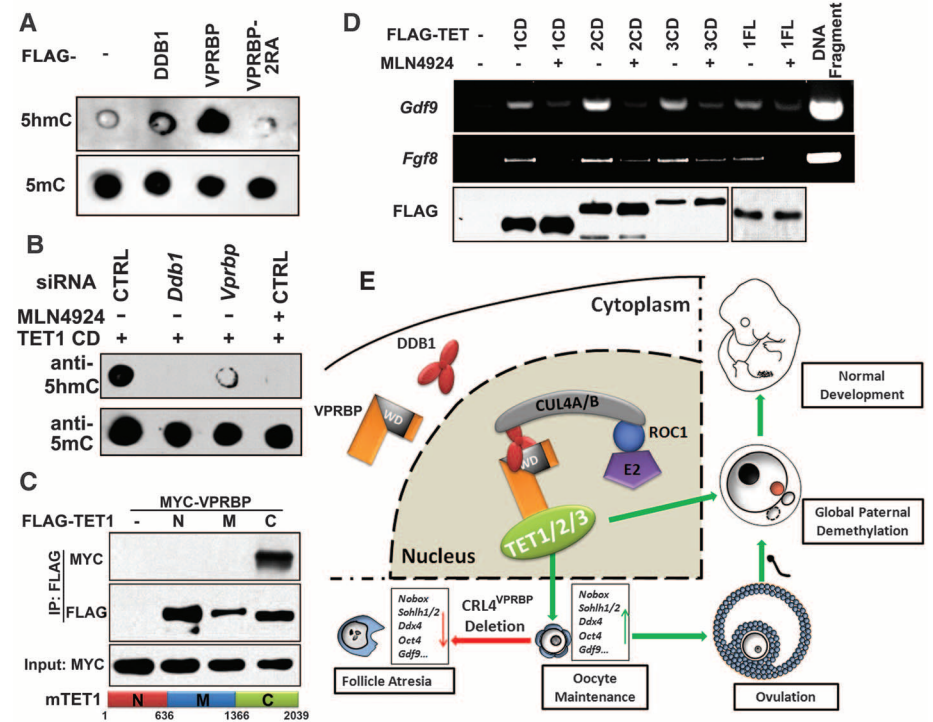


Fig. 4. CRL4^{VPRBP} binds to TET enzymes and regulates their activities. (A) Dot blotting results showing endogenous 5hmC levels after DDB1 or VPRBP overexpression. (B) Dot blotting results showing that TET1 overexpression increased 5hmC levels in HeLa cells; this effect was abolished by both *Ddb1* or *Vprbp* depletion and MLN4924 treatment. (C) Coimmunoprecipitation results showing the interactions between VPRBP and TET1-CD. (D) Impaired DNA binding of TET1/2/3-CD and TET1-FL (full length) after CRL4 inhibition. FLAG tagged TET1/2/3-CD and TET1-FL were expressed and/or purified with or without MLN4924 and were incubated with DNA fragments amplified from *Gdf9* and *Fgf8* promoters. The TET-bound DNA fragments were detected by PCR and agarose electrophoresis. (E) Illustration of CRL4^{VPRBP} functions in mammalian oocytes. CRL4^{VPRBP} enters the nucleus and activates TETs, which convert 5mC to 5hmC, regulate DNA methylation levels, and maintain the expressions of genes essential for oocyte survival in primordial follicles. When grown oocytes are ovulated and fertilized, CRL4^{VPRBP} recruits TET3 into the male pronucleus and activates zygotic genome reprogramming which is essential for embryo development. WD, WD40.

developmental potential, even before their physical disappearance. The role of CRL4^{VPRBP} in mammalian oocytes is summarized in Fig. 4E.

Although CRL4^{VPRBP} is crucial for TET activities, our results suggest that TET1, 2, and 3 are not the only CRL4^{VPRBP} substrates in oocytes. More than one-third of the embryos derived from TET3-deleted oocytes could develop to term (*11*). However, all embryos that were derived from DDB1-deleted oocytes died before the eight-cell stage, which indicated that they had defects other than TET3-mediated genome reprogramming. CRL4 might also recruit other substrate adaptors, poly-ubiquitinate a number of protein substrates, and direct them toward degradation. Identifying other CRL4^{VPRBP} substrates will shed new light on the molecular regulatory mechanisms of oocyte functions.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S11

Tables S1 to S3

References (15–24)

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Serial Femtosecond Crystallography of G Protein–Coupled Receptors

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X-ray crystallography of G protein–coupled receptors and other membrane proteins is hampered by difficulties associated with growing sufficiently large crystals that withstand radiation damage and yield high-resolution data at synchrotron sources. We used an x-ray free-electron laser (XFEL) with individual 50-femtosecond-duration x-ray pulses to minimize radiation damage and obtained a high-resolution room-temperature structure of a human serotonin receptor using sub-10-micrometer microcrystals grown in a membrane mimetic matrix known as lipidic cubic phase. Compared with the structure solved by using traditional microcrystallography from cryo-cooled crystals of about two orders of magnitude larger volume, the room-temperature XFEL structure displays a distinct distribution of thermal motions and conformations of residues that likely more accurately represent the receptor structure and dynamics in a cellular environment.

Gprotein–coupled receptors (GPCRs) represent a highly diverse superfamily of eukaryotic membrane proteins that mediate cellular communication. In humans, ~800 GPCRs respond to a variety of extracellular signaling mol-

ecules and transmit signals inside the cell by coupling to heterotrimeric G proteins and other effectors. Their involvement in key physiological and sensory processes in humans makes GPCRs prominent drug targets. Despite the high biomedical relevance and decades of dedicated research, knowledge of the structural mechanisms of ligand recognition, receptor activation, and signaling in this broad family remains limited. Challenges for GPCR structural studies include low-expression yields, low receptor stability after detergent extraction from native membranes, and high conformational heterogeneity. Many years of developments aimed at receptor stabilization, crystallization, and microcrystallography culminated in a series of breakthroughs in GPCR structural biology leading to the structure determination of 22 receptors, some of which were solved in several conformational states and one in complex with its G protein partner (*1–5*).

Nonetheless, crystallographic studies of GPCRs remain difficult because many of them produce only microcrystals. Most GPCR structures to date have been obtained by using crystallization from the membrane-mimetic environment of a lipidic cubic phase (LCP) (*6, 7*). LCP crystallization has proven successful for obtaining high-resolution structures of a variety of membrane proteins, including ion channels, transporters, and enzymes, in addition to GPCRs (*8, 9*). This method leads to highly ordered crystals that are, however, often limited in size. Microfocus x-ray beams of high intensity (~10⁹ photons/s/μm²) and long exposures (~5 s) are typically required in order to obtain sufficient intensity for high-resolution data from weakly diffracting microcrystals. The high-radiation doses induce severe radiation damage and require merging data from multiple crystals in order to obtain complete data sets of sufficient quality. Accordingly, sub-10-μm GPCR crystals are currently not suitable for high-resolution data collection, even at the most powerful synchrotron microfocus beamlines (*7, 10*).

Serial femtosecond crystallography (SFX) (*11*), which takes advantage of x-ray free-electron lasers (XFEL), has recently demonstrated great promise for obtaining room-temperature high-resolution data from micrometer- and sub-micrometer-size crystals of soluble proteins, with minimal radiation damage (*12, 13*). The highly intense (~2 mJ, 10¹² photons per pulse) and ultrashort (<50 fs) x-ray pulses produced by XFELs enable the recording of high-resolution diffraction snapshots from individual crystals at single orientations before their destruction. SFX data collection, therefore, relies on a continuous supply of small crystals intersecting the XFEL beam in random orientations—typically provided by a fast-running liquid microjet (*12*)—which is incompatible with streaming highly viscous gel-like materials such as LCP and requires tens to hundreds of milligrams of crystallized protein for data collection (*11*). For many membrane proteins, including most human membrane proteins, obtaining such quantities is not practical.

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We have modified the SFX data collection approach (Fig. 1) and obtained a room-temperature GPCR structure at 2.8 Å resolution using only 300 µg of protein crystallized in LCP. SFX experiments were performed at the Coherent X-ray Imaging (CXI) instrument of the Linac Coherent Light Source (LCLS) (14). LCP-grown microcrystals (average size of 5 by 5 by 5 µm) (fig. S1) (15) of the human serotonin 5-HT_{2B} receptor (16) bound to the agonist ergotamine were continuously delivered across a ~1.5-µm-diameter XFEL beam by using a specially designed LCP injector. LCP with randomly distributed crystals was ex-

truded through a 20- to 50-µm capillary into a vacuum chamber (10^{-4} torr) at room temperature (21°C) (17) and a constant flow rate of 50 to 200 nL/min and was stabilized by a co-axial flow of helium or nitrogen gas supplied at 20 to 30 bar. We recorded single-pulse diffraction patterns (fig. S2) using 9.5-keV (1.3 Å) x-ray pulses of 50 fs duration at a 120 Hz repetition rate by means of a Cornell-SLAC pixel array detector (CSPAD) (18) positioned at a distance of 100 mm from the sample. The XFEL beam was attenuated to 3 to 6% so as to avoid detector saturation. The average x-ray pulse energy at the sample was 50 µJ

(3×10^{10} photons/pulse), corresponding to a radiation dose of up to 25 megagrays per crystal. A total of 4,217,508 diffraction patterns were collected within 10 hours by using ~100 µL of crystal-loaded LCP, corresponding to ~0.3 mg of protein. Of these patterns, 152,651 were identified as crystal hits (15 or more Bragg peaks) by the processing software Cheetah (<http://www.desy.de/~barty/cheetah/>), corresponding to a hit rate of 3.6%. Of these crystal hits, 32,819 patterns (21.5%) were successfully indexed and integrated by CrystFEL (19) at 2.8 Å resolution (table S1). The structure was determined through molecular replacement and refined to $R_{\text{work}}/R_{\text{free}} = 22.7/27.0\%$. Overall, the final structure (fig. S3) has a well-defined density for most residues, including the ligand ergotamine (fig. S4).

We compared the XFEL structure of the 5-HT_{2B} receptor/ergotamine complex (5-HT_{2B}-XFEL) with the recently published structure of the same receptor/ligand complex obtained by means of traditional microcrystallography at a synchrotron source [Protein Data Bank (PDB) ID 4IB4; 5-HT_{2B}-SYN] (21). Synchrotron data were collected at 100 K on cryo-cooled crystals of a much larger size (average volume, $\sim 10^4$ µm³) than those used for the XFEL structure (average volume, $\sim 10^2$ µm³) (fig. S1). Other differences between data collection protocols are listed in table S1. Both data sets were processed in the same spacegroup C221₁, which is expected given the very similar crystallization conditions. However, the lattice parameters for the room-temperature XFEL crystals are slightly longer in the *a* and *b* directions and slightly shorter in the *c* direction, resulting in a 2.1% larger unit cell volume. Concomitant with these lattice changes, we observed a ~2.5° rotation of

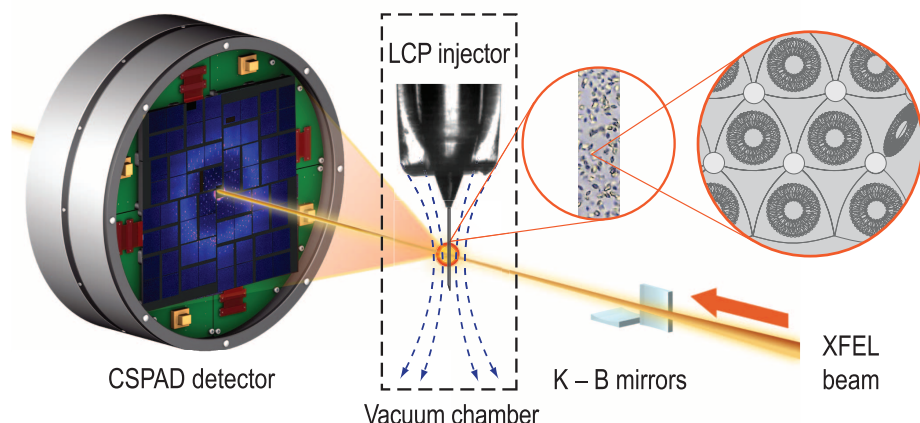
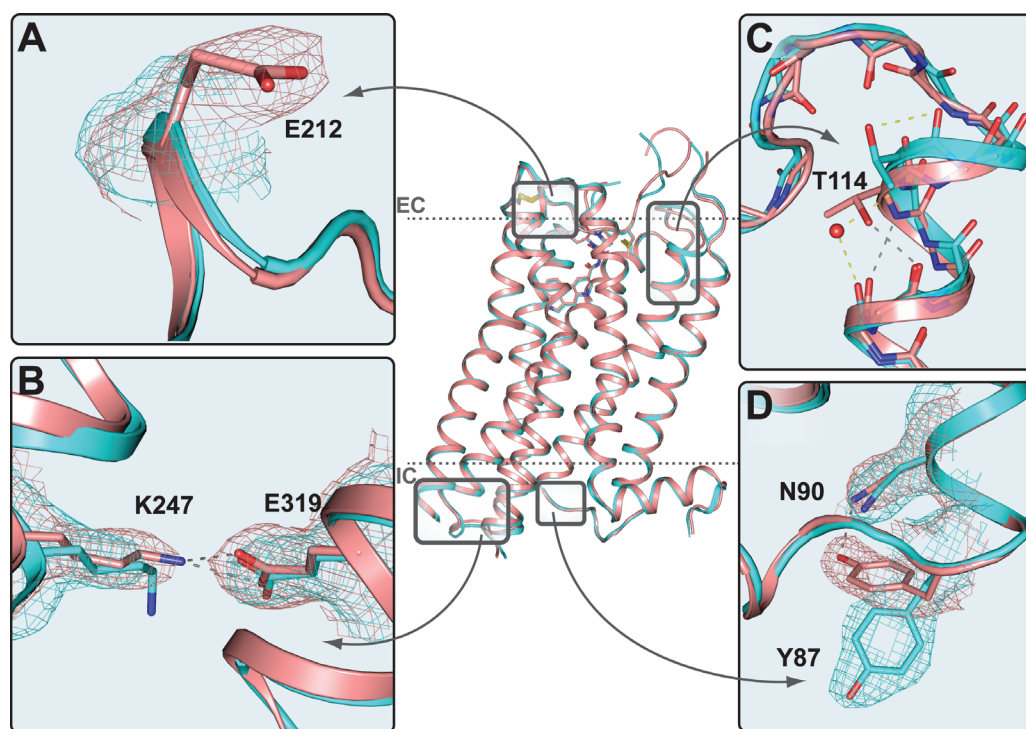


Fig. 1. Experimental setup for SFX data collection using an LCP injector. 5-HT_{2B} receptor microcrystals (first zoom level) dispersed in LCP (second zoom level) are injected as a continuous column of 20 to 50 µm in diameter—stabilized by a co-axial gas flow (blue dash curved lines)—inside a vacuum chamber and intersected with 1.5-µm-diameter pulsed XFEL beam focused with Kirkpatrick-Baez (K-B) mirrors. Single-pulse diffraction patterns were collected at 120 Hz by using a CSPAD detector. The entire XFEL beam path and CSPAD are under vacuum.

Fig. 2. Comparison between 5-HT_{2B}-XFEL (light red) and 5-HT_{2B}-SYN (teal) structures. Central image represents a backbone overlay of the two structures. Dashed lines correspond to membrane boundaries defined by the Orientation of Proteins in Membrane database (<http://opm.phar.umich.edu>) (28). (A) Electron density for the Glu212 side chain is missing in 5-HT_{2B}-SYN and fully resolved in 5-HT_{2B}-XFEL. (B) A salt bridge between Glu319 and Lys247 links intracellular parts of helices V and VI in the 5-HT_{2B}-XFEL structure. In the 5-HT_{2B}-SYN structure, Lys247 makes a hydrogen bond with Tyr1105 from the BRIL fusion protein. (C) Extracellular tip of helix II forms a regular helix in 5-HT_{2B}-XFEL with Thr114, making a stabilizing hydrogen bond with the backbone carbonyl, whereas in 5-HT_{2B}-SYN, a water-stabilized kink is introduced at this position. (D) Tyr87 forms a hydrogen bond with Asn90 in 5-HT_{2B}-XFEL; this hydrogen bond is broken, and Tyr87 adopts a different rotamer conformation in the 5-HT_{2B}-SYN structure. 2mF_{obs}-DF_{calc} maps (contoured at 1σ level) are shown only around described residues.



the b₅₆₂ RIL (BRIL) fusion domain with respect to the receptor (fig. S5). Otherwise, the receptor domains of the 5-HT_{2B}-XFEL and 5-HT_{2B}-SYN structures are very similar [receptor C α root mean square deviation (RMSD) = 0.46 Å, excluding flexible residues at the N terminus, 48 to 51, and in the extracellular loop 2 (ECL2), 195 to 205] (Fig. 2). The ligand ergotamine has indistinguishable electron density and placement (total ligand RMSD = 0.32 Å) in both structures (Fig. 2 and fig. S4B). The largest backbone deviations were observed in the loop regions, especially in the stretch of ECL2 between helix IV and the Cys128–Cys207 disulfide bond, which is apparently very flexible. We observed an unexpected backbone deviation at the extracellular tip of helix II (Fig. 2C), which adopts a regular α -helix in the 5-HT_{2B}-XFEL structure, with Thr¹¹⁴ forming a stabilizing hy-

drogen bond with the main chain carbonyl of Ile110. In the 5-HT_{2B}-SYN structure, however, a water-stabilized kink was found at this location, which results in the two structures deviating by 2.0 Å (at C α atom of Thr114) at the tip of helix II and up to 3.4 Å (at O atom of Phe117) in ECL1.

Although absolute B- (or temperature) factor values can be affected by errors associated with experimental conditions, their distribution generally represents the relative static and dynamic flexibility of the protein in the crystal (22). Because both structures were obtained from similar samples and at similar resolutions, we analyzed their B-factor distributions so as to study the effect of the different temperatures on the thermal motions of the receptor. The average B-factor for the receptor part in the room-temperature 5-HT_{2B}-XFEL structure (88.4 Å²) is 21 Å² larger than that in the

cryo 5-HT_{2B}-SYN structure (67.2 Å²), which is consistent with larger thermal motions at higher temperature and possible effects of Bragg termination during the XFEL pulse (20). The distribution of B-factors highlights a more rigid core of the seven transmembrane helices in comparison with loops, with more pronounced B-factor deviations observed in the room-temperature 5-HT_{2B}-XFEL structure (Fig. 3 and fig. S6). N terminus, intracellular loop 2 (ICL2), ECL1, and part of ECL2 between helix IV and the Cys128–Cys207 disulfide bond show much larger deviations in B-factors (50 to 100 Å²) between the two structures as compared with the average difference of 21 Å². These parts of the structure are not involved in direct interactions with the ligand ergotamine, but their mobility may affect the kinetics of ligand binding and interactions with intracellular binding partners (23). In contrast, ICL1, part of ECL2 between the Cys128–Cys207 disulfide bond and helix V, and ECL3 display just an average increase in the B-factors, suggesting that the relative range of their thermal fluctuations was adequately captured in the cryo structure. As previously established with cryocrystallography, one of the most pronounced differences between the two subtypes of serotonin receptors, 5-HT_{2B} and 5-HT_{1B}, occurs at the extracellular tip of helix V and ECL2, which forms an additional helical turn stabilized by a water molecule in 5-HT_{2B} (21). This additional turn pulls the extracellular tip of helix V toward the center of the helical bundle and was suggested to be responsible for the biased agonism of ergotamine at the 5-HT_{2B} receptor. The 5-HT_{2B}-XFEL structure confirms the rigid structured conformation of ECL2, stabilized by a comprehensive network of hydrogen bonds, involving residues Lys193, Glu196, Arg213, Asp216, and a lipid OLC (monoolein) (fig. S7); however, no ordered water molecule was observed, emphasizing that water is more disordered and probably does not play a substantial structural role at this location.

Several side chains have partly missing electron density in both room-temperature and cryo structures (table S2). Such lack of density is most likely related to disorder of the corresponding side chains (such as residues at the N terminus, ECL2, and ICL2) (Fig. 2A). Two disulfide bonds, Cys128–Cys207 and Cys350–Cys353, are intact and well resolved in both structures; however, the B-factor increase in the 5-HT_{2B}-XFEL structure compared with 5-HT_{2B}-SYN for each of these disulfide bonds (11.1 and 5.7 Å², respectively) is lower than that of the average B-factor increase (21 Å²). Several side chains have different rotamer conformations between the two structures (Fig. 2D and table S3), which is consistent with a partial remodeling of the side chain conformational distribution upon cryo-cooling observed in soluble proteins (24). Several interactions involving charged residues appear stronger and better defined in 5-HT_{2B}-XFEL compared with the 5-HT_{2B}-SYN structure (table S4). This strengthening of the charged interactions at higher temperatures potentially can be explained

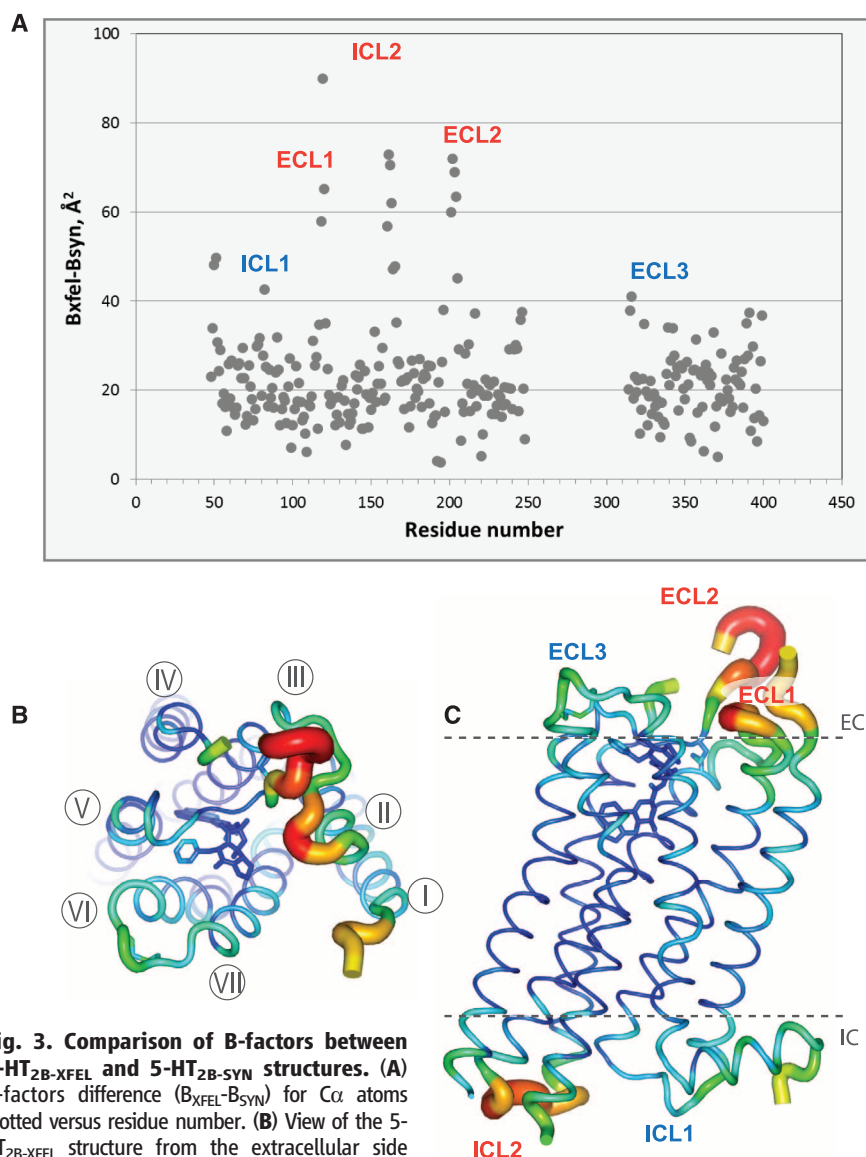


Fig. 3. Comparison of B-factors between 5-HT_{2B}-XFEL and 5-HT_{2B}-SYN structures. (A) B-factors difference ($B_{\text{XFEL}} - B_{\text{SYN}}$) for C α atoms plotted versus residue number. (B) View of the 5-HT_{2B}-XFEL structure from the extracellular side and (C) in the lateral-to-membrane orientation. Structure in (B) and (C) is shown in putty representation and colored in rainbow colors by the C α B-factors (range 60 to 170 Å²). Loops for which the B-factor difference is above 50 Å² are labeled in red, and those with a difference below 50 Å² are in blue in (A) and (C). Helices are labeled in (B).

by a decrease in the dielectric constant of water with temperature, reducing the desolvation penalty (25, 26). In particular, the salt bridge between Glu319 and Lys247 is well defined in the 5-HT_{2B}-XFEL structure but appears broken in the cryo 5-HT_{2B}-SYN structure (Fig. 2B). Because GPCR activation has been associated with large-scale structural changes in the intracellular parts of helices V and VI, this salt bridge may play a role in the receptor function and is likely to be more accurately resolved and represented in the 5-HT_{2B}-XFEL structure recorded at room temperature.

Overall, the observed differences likely originate from effects related to thermal motions, cryo-cooling (24), and radiation damage (27). Thus, the XFEL source enables access to a room-temperature GPCR structure, which more accurately represents the conformational ensemble for this receptor under native conditions. Because dynamics are an integral part of GPCR biology, the use of SFX to accurately determine GPCR structural details at room temperature can make an important contribution to understanding the structure-function relationships in this superfamily.

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15. Materials and methods are available as supplementary materials on Science Online.
16. The engineered-for-crystallization construct is based on the sequence of the human 5-HT_{2B} receptor with the following modifications: (i) Residues Tyr249–Val313 within ICL3 were replaced with Ala1–Leu106 of thermostabilized apo cytochrome BRIL; (ii) N-terminal residues 1 to 35 and C-terminal residues 406 to 481 were truncated; and (iii) a thermostabilizing Met144 Trp mutation was introduced.
17. The temperature measured in the CXI hutch during the experiments was 294 K (21°C). The actual crystal temperature was likely a few degrees lower because of the evaporative cooling upon injection of crystal-loaded LCP in vacuum.
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microcrystals in LCP and helped with data collection, structure refinement, analysis, and writing the paper. C.G. participated in data collection and processed and analyzed data. G.W.H. performed structure refinement. D.J., Di.W., and G.N. helped develop and operate the LCP injector. U.W. conceived, designed, and developed the LCP injector. V.K. analyzed the results and helped with writing the paper. A.B. participated in data collection, wrote data processing software, and helped with data processing and writing the paper. N.A.Z. and Sh.B. participated in data collection and helped with data processing. D.L. helped with data collection. Se.B., M.M., G.J.W., J.E.K., and M.M.S. set up the XFEL experiment, beamline, controls, and data acquisition; operated the CXI beamline; and performed the data collection. C.W. helped with sample preparation. S.T.A.S. synthesized and purified 7.9 MAG. R.F., C.K., K.N.R., and I.G. participated in data collection and contributed to sample characterization. P.F. was involved in the initiation and planning of the experiments, assisted with sample characterization and data collection, and contributed to writing the paper. R.A.K. developed the Monte Carlo integration method and contributed to data processing. K.R.B. contributed to software development and data processing. T.A.W. developed the Monte Carlo integration method, wrote data processing software, and contributed to data processing. H.N.C. supervised software development and data processing and helped with writing the paper. M.C. provided the 7.9 MAG and helped with data collection and with writing the paper. J.C.H.S. helped develop the LCP injector and developed the Monte Carlo integration method with R.A.K. R.C.S. supervised GPCR production and contributed to writing the paper. V.C. conceived the project, designed the experiments, supervised data collection, performed structure refinement, analyzed the results, and wrote the paper.

Supplementary Materials

www.sciencemag.org/content/342/6165/1521/suppl/DC1
Materials and Methods
Figs. S1 to S8
Tables S1 to S4
References (29–41)

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mTOR Inhibition Alleviates Mitochondrial Disease in a Mouse Model of Leigh Syndrome

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Mitochondrial dysfunction contributes to numerous health problems, including neurological and muscular degeneration, cardiomyopathies, cancer, diabetes, and pathologies of aging. Severe mitochondrial defects can result in childhood disorders such as Leigh syndrome, for which there are no effective therapies. We found that rapamycin, a specific inhibitor of the mechanistic target of rapamycin (mTOR) signaling pathway, robustly enhances survival and attenuates disease progression in a mouse model of Leigh syndrome. Administration of rapamycin to these mice, which are deficient in the mitochondrial respiratory chain subunit Ndufs4 [NADH dehydrogenase (ubiquinone) Fe-S protein 4], delays onset of neurological symptoms, reduces neuroinflammation, and prevents brain lesions. Although the precise mechanism of rescue remains to be determined, rapamycin induces a metabolic shift toward amino acid catabolism and away from glycolysis, alleviating the buildup of glycolytic intermediates. This therapeutic strategy may prove relevant for a broad range of mitochondrial diseases.

Leigh syndrome is a clinically defined disease resulting from genetic defects that disrupt mitochondrial function. It is the

most common childhood mitochondrial disorder, affecting 1 in 40,000 newborns in the United States (*1*). Leigh syndrome is characterized by

retarded growth, myopathy, dyspnea, lactic acidosis, and progressive encephalopathy primarily in the brainstem and basal ganglia (2, 3). Patients typically succumb to respiratory failure from the neuropathy, with average age of death at 6 to 7 years (1).

We recently observed that reduced nutrient signaling, accomplished by glucose restriction or genetic inhibition of mTOR, is sufficient to rescue short replicative life span in several budding yeast mutants defective for mitochondrial function (4), including four mutations associated with human mitochondrial disease (fig. S1). These observations led us to examine the effects of rapamycin, a specific inhibitor of mTOR, in a mammalian model of Leigh syndrome, the *Ndufs4* knockout (*Ndufs4*^{-/-}) mouse (5). *Ndufs4* encodes

a protein involved in assembly, stability, and activity of complex I of the mitochondrial electron transport chain (ETC) (6, 7). *Ndufs4*^{-/-} mice show a progressive neurodegenerative phenotype characterized by lethargy, ataxia, weight loss, and ultimately death at a median age of 50 days (5, 8). Neuronal deterioration and gliosis closely resemble the human disease, with primary involvement of the vestibular nuclei, cerebellum, and olfactory bulb.

We first examined the effects of delivering rapamycin (8 mg/kg) every other day by intraperitoneal injection beginning at weaning [approximately postnatal day 20 (P20)]. This treatment reduces mTOR signaling in wild-type mice (9) and provided significant increases in median survival of male (25%) and female (38%) knockout mice (Fig. 1A). A slight reduction in maximum body size and a delay in age of disease onset were also observed (Fig. 1B and fig. S2). Although these results showed that *Ndufs4*^{-/-} mice benefit from rapamycin treatment, we noted that by 24 hours after injection, rapamycin levels in blood were reduced by more than 95% (fig. S3). We therefore performed a follow-up study de-

livering rapamycin (8 mg/kg) daily by intraperitoneal injection starting at P10, which resulted in blood levels ranging from >1800 ng/ml immediately after injection to 45 ng/ml trough levels (fig. S3). For comparison, an encapsulated rapamycin diet that extends life span in wild-type mice by about 15% achieves steady-state blood levels of about 60 to 70 ng/ml, and trough levels between 3 and 30 ng/ml are recommended for patients receiving rapamycin (10). In the daily-treated cohort, we observed a striking extension of median and maximum life span; the longest-lived mouse survived 269 days. Median survival of males and females was 114 and 111 days, respectively (fig. S2C).

Vehicle-injected knockout mice first displayed neurological symptoms around P35, coinciding with a body weight peak (Fig. 1, B to D, and fig. S2D). After this point, disease symptoms progressively worsened and weight declined. Daily rapamycin treatment dampened developmental weight gain and prevented the progressive weight loss phenotype (Fig. 1B and fig. S2E). This effect was robust, even among mice from the same litter (fig. S4). Incidence and severity of clasp-

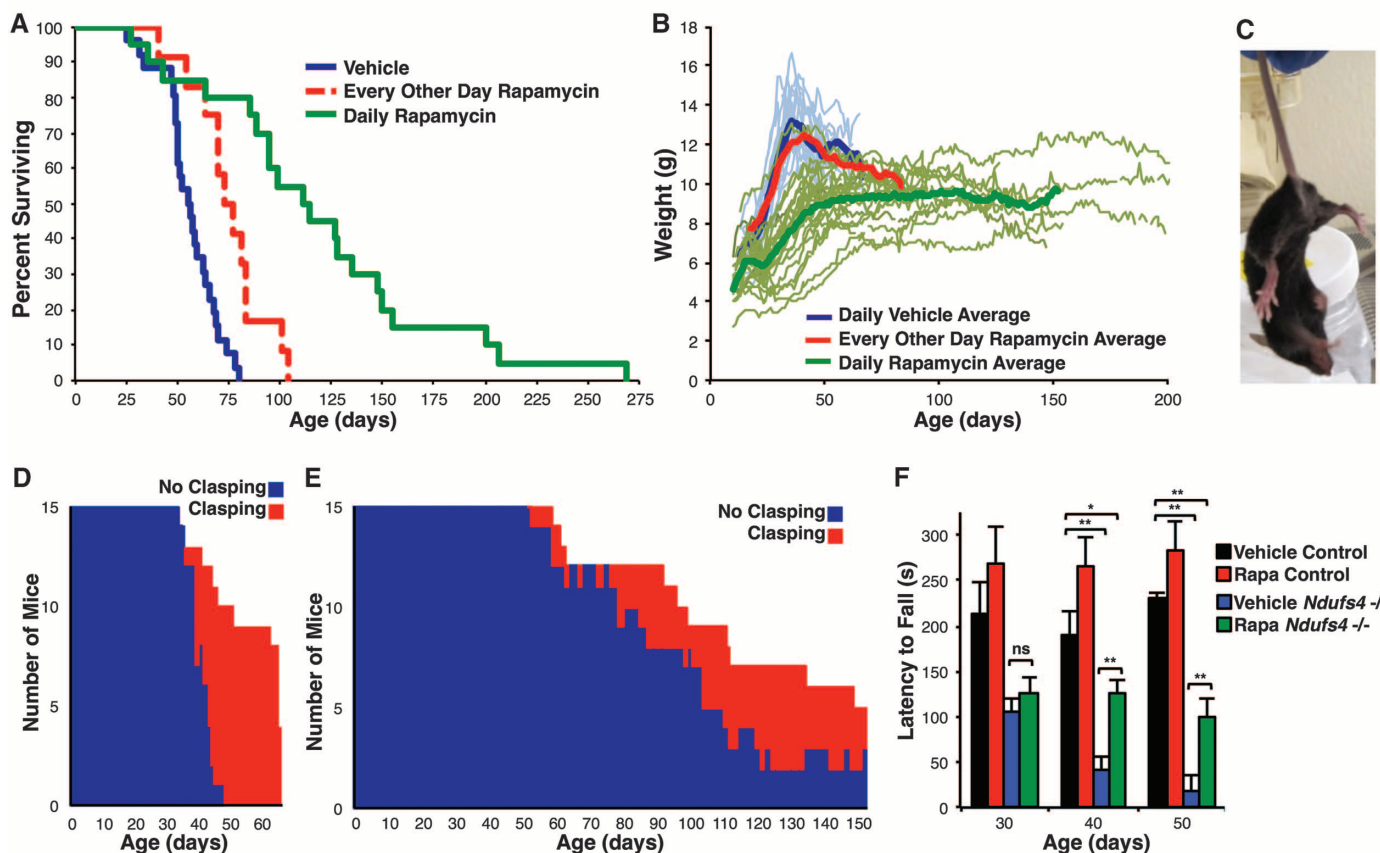


Fig. 1. Reduced mTOR signaling improves health and survival in a mouse model of Leigh syndrome. (A) Survival of the *Ndufs4*^{-/-} mice was significantly extended by rapamycin injection every other day; life span more than doubled with daily rapamycin treatment (log-rank $P = 0.0002$ and $P < 0.0001$, respectively). (B) Body weight plots of *Ndufs4*^{-/-} mice. (C) Representative forelimb clasp behavior, a widely used sign of neurological degeneration. Clasp involves an inward curling of the spine and a retraction of forelimbs (shown here)

or all limbs toward the midline of the body. (D and E) Clasp in vehicle-treated (D) and daily rapamycin-treated (E) *Ndufs4*^{-/-} mice as a function of age. A total of 15 mice were observed for clasp for each treatment. Age of onset of clasp behavior is significantly delayed in rapamycin-treated mice ($**P < 0.001$ by log-rank test). (F) *Ndufs4*^{-/-} mice show a progressive decline in rotarod performance that is rescued by rapamycin ($*P < 0.05$, $**P < 0.005$, Student's t test; error bars are $\pm$ SEM). (See also fig. S5, which indicates replicate numbers.)

a commonly reported and easily scored phenotype that progresses with weight loss and neurological decline, was also greatly attenuated in rapamycin-treated knockouts (Fig. 1, C to E). Performance in a rotarod assay, which measures balance, coordination, and endurance, was assessed in a separate cohort of mice. Vehicle-treated knockout mouse performance worsened as the disease progressed, whereas rapamycin-treated knockout mice maintained their performance with age (Fig. 1F and fig. S5). Dyspnea, previously observed in vehicle-treated knockout mice (5), was not observed in the mice injected daily with rapamycin.

Rapamycin-treated knockout mice also did not develop the neurological lesions associated with this disease (red arrows in Fig. 2, A and B; see also fig. S6). The lesions, characterized by astrocyte activation and glial reactivity [detected by glial fibrillary acidic protein (GFAP) and Iba1 staining, respectively] were detectable in all vehicle-treated knockout mice over 50 days of age (5). We were unable to detect lesions in the cerebellum of age-matched rapamycin-treated knockout mice. GFAP, Iba1, and laminin (a marker of neovascularization) were markedly increased in olfactory bulbs of vehicle-treated but not in rapamycin-treated knockout mice (Fig. 2B and fig. S6). Rapamycin did not affect GFAP, Iba1,

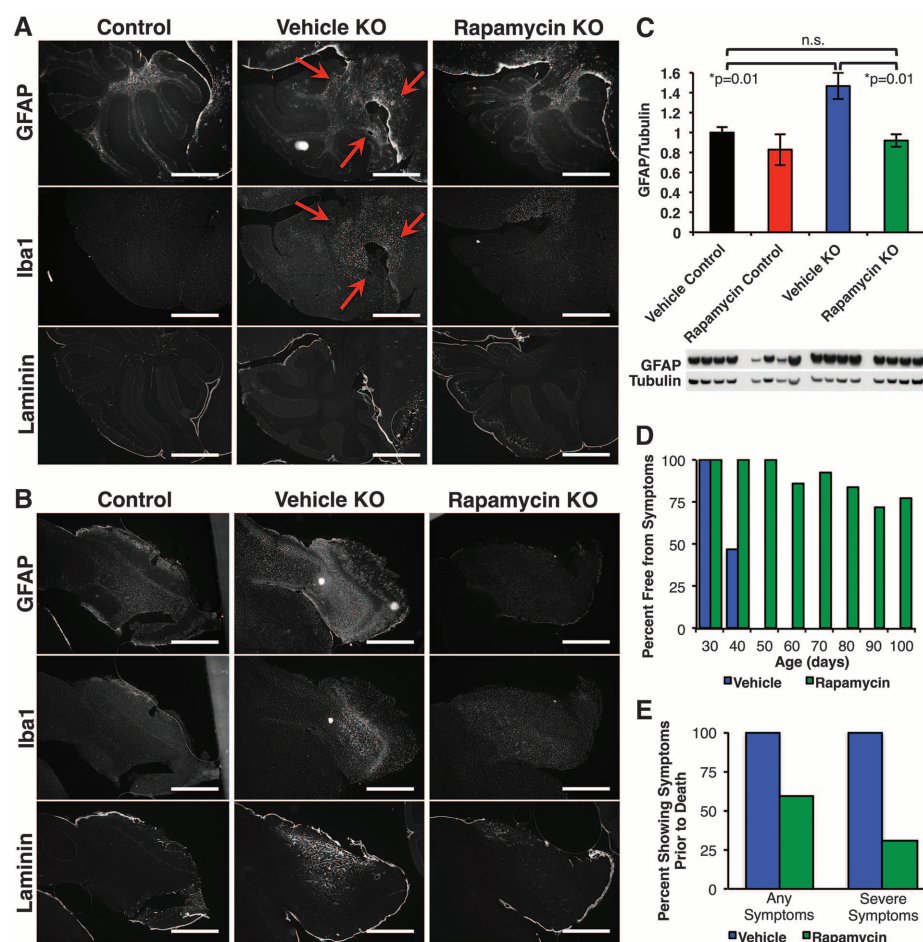
or laminin levels in wild-type mice (fig. S6). Western blotting for GFAP using whole-brain lysates from ~50-day-old mice revealed a significant increase in GFAP in knockout mice that was attenuated by rapamycin (Fig. 2C). Furthermore, no lesions were detected in 100-day-old and 268-day-old rapamycin-treated knockout mice (fig. S7). Overall, the percentage of mice showing neurological symptoms was much reduced at every age point after P35 in rapamycin-treated knockout mice, with about half never exhibiting overt signs of neurological disease before death (Fig. 2, D and E).

Given the pleiotropic effects of mTOR inhibition [reviewed in (11, 12)], we sought to identify downstream mechanisms associated with attenuation of mitochondrial disease. Rapamycin has well-documented immune-modulatory effects, so we first considered that the benefit might arise from reduced neuroinflammation. To test this model, we treated mice with FK-506 (tacrolimus), a clinically approved immunosuppressive drug that binds the same target as rapamycin, FKBP12, but inhibits calcineurin signaling rather than mTOR (13). FK-506 did not affect disease onset or progression (Fig. 3, A and B, and fig. S8), indicating that neither immunosuppression nor off-target disruption of calcineurin by binding of FKBP12 are likely to account for the effects of rapamycin. We

next considered that rapamycin might improve mitochondrial function by increasing macroautophagy, removing the least functional components of the mitochondrial network. Although we were able to detect evidence of induction of autophagy in the liver and brain of rapamycin-treated mice (fig. S9), there was no corresponding rescue of mitochondrial function (Fig. 3C and fig. S10). Complex I assembly and stability, assessed by blue native gel electrophoresis, were also unaltered by rapamycin (Fig. 3D and fig. S11), as were levels and localization of ETC proteins (Fig. 3, E and F, and fig. S12). We found no evidence for induction of HSP60, a component of the mitochondrial unfolded protein response, either by NDUFS4 loss or by rapamycin (Fig. 3F and fig. S12).

As a central coordinator of nutrient sensing and growth, mTOR regulates metabolism by integrating levels of amino acids at the lysosome, energetic sensing by adenosine monophosphate-activated protein kinase (AMPK), and extracellular signals through insulin and insulin-like growth factor (IGF) (11, 14). We reasoned that loss of NDUFS4 might perturb metabolic signaling and affect mTOR activity. Consistent with this idea, phosphorylation of ribosomal protein S6, a target of mTOR complex 1 (mTORC1) signaling, was significantly increased in the brains of

Fig. 2. Rapamycin reduces neurological disease in *Ndufs4*^{-/-} mice. (A) Representative cerebellar staining for neurological lesions in 55- to 60-day-old mice. All vehicle-treated mice showed glial activation and lesions at this age, whereas lesions were not detected in age-matched daily rapamycin-treated mice ($n = 6$; scale bars, ~500 μm) (see also figs. S6 and S7). (B) Representative olfactory bulb staining shows activation of glia by GFAP staining and neovascularization by laminin staining in vehicle-treated knockout (KO) mice and a robust attenuation in rapamycin-treated KO mice ($n = 6$ per treatment; scale bars, ~500 μm). (C) Western blotting of whole-brain lysates from a separate cohort of mice shows increased GFAP in vehicle KO mice and rescue to control levels by rapamycin ($*P < 0.05$, Student's t test; error bars are $\pm$ SEM). (D and E) The percentage of living mice showing neurological symptoms is greatly reduced by daily rapamycin treatment (D), as is the number of mice showing neurological symptoms at the time of death (E).



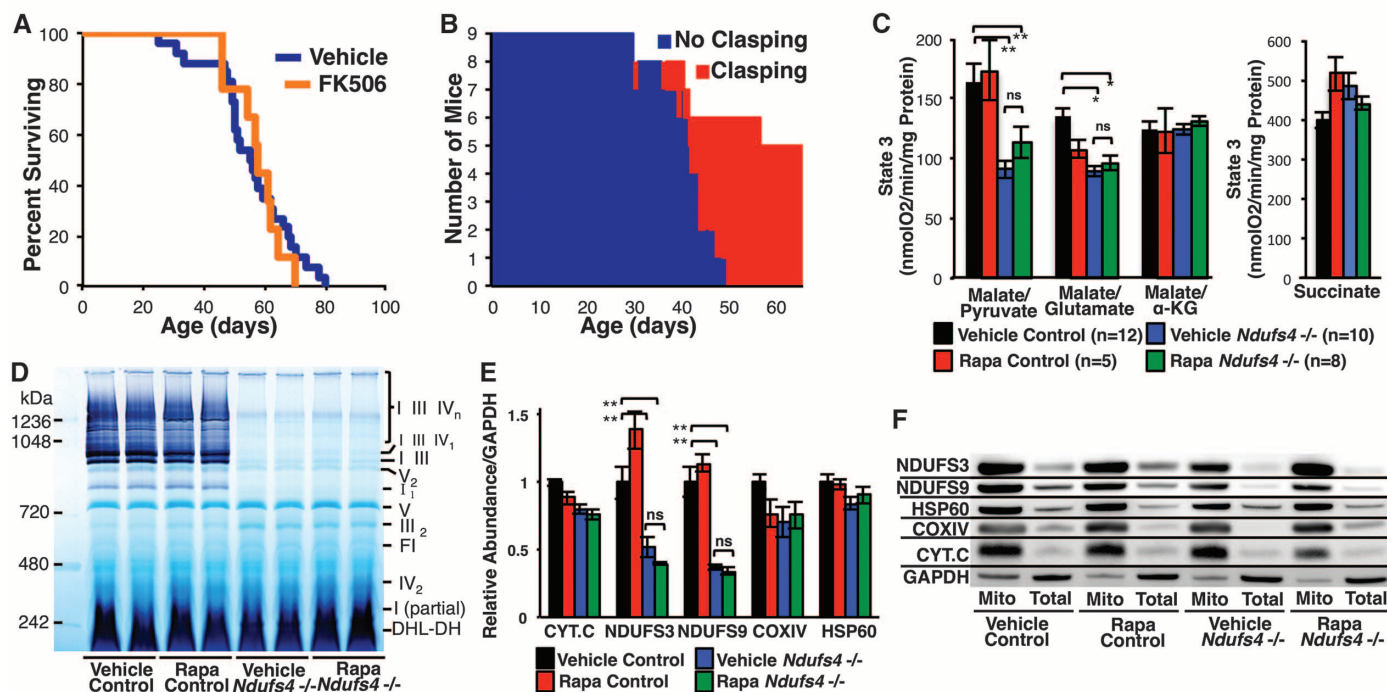


Fig. 3. Rapamycin does not substantially alter mitochondrial function or complex I assembly. (A and B) FK-506 delivered at the highest tolerated dose (see fig. S8) failed to enhance survival (A) or attenuate disease (B) in *Ndufs4*^{-/-} mice. (C) Rapamycin has no observed effect on respiratory activity or complex I deficiency of mitochondria isolated from ~50-day-old *Ndufs4*^{-/-} mice; *n* = 4 to 6 mice per data point. (see also fig. S10). (D) Native-in-gel activity assays reveal that rapamycin does not influence assembly or sta-

bility of complex I (see also fig. S11). (E and F) Complex I subunits (NDUFS3 and NDUFS9) are significantly reduced in *Ndufs4*^{-/-} mice, and rapamycin has no effect on their total levels (F) or subcellular localization (E) in brain. Levels of other mitochondrial proteins (cytochrome c, the complex IV subunit COXIV, and HSP60) are independent of both *Ndufs4* genotype and treatment (see also fig. S9). **P* < 0.05, ***P* < 0.005, Student's *t* test; ns, not significant. Error bars are $\pm$ SEM.

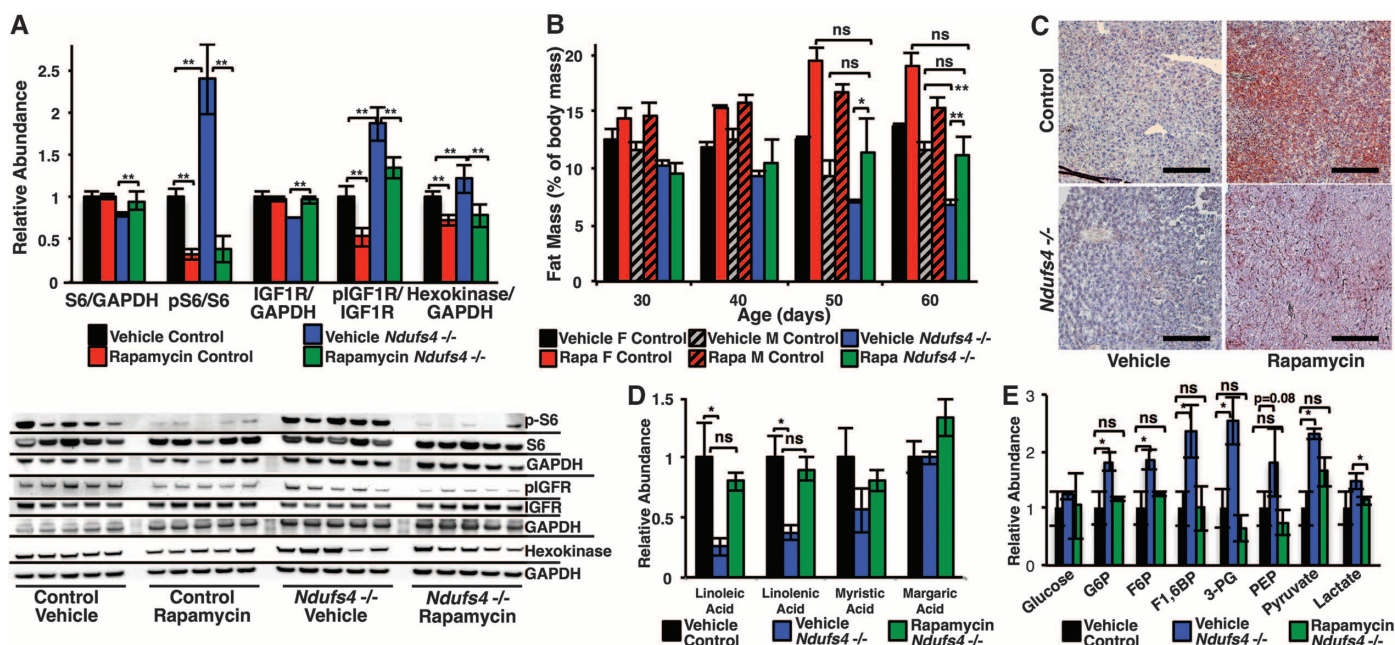


Fig. 4. *Ndufs4*^{-/-} mice exhibit mTOR activation and metabolic defects that are suppressed by rapamycin. (A) mTOR activity, as indicated by phosphorylation of S6, is increased in *Ndufs4*^{-/-} mouse brain. Total IGF1R and S6 are decreased in *Ndufs4*^{-/-} mice, suggesting feedback inhibition from chronic mTOR activation. Rapamycin potentially inhibits phosphorylation of S6 and rescues levels of IGF1R and S6. (B) Total body fat progressively decreases in *Ndufs4*^{-/-} mice but is maintained in rapamycin-treated mice. Fat mass differs by sex in control but not

Ndufs4^{-/-} mice (*n* = 4 to 6 mice per data point). (C and D) Liver fat droplets are deficient in vehicle-treated *Ndufs4*^{-/-} mice and partially rescued by rapamycin (representative images, *n* > 6 stained per treatment; scale bar, ~100 μ m) (C), as are free fatty acids detected by metabolomics (D) (*n* = 4 per treatment). (E) Accumulation of glycolytic intermediates in *Ndufs4*^{-/-} brain is suppressed by rapamycin (*n* = 4 per treatment) (see fig. S14 and tables S1 to S3). **P* < 0.05, ***P* < 0.005, Student's *t* test; error bars are $\pm$ SEM.

knockout mice (Fig. 4A), and rapamycin reduced phosphorylation of S6 in both wild-type and knockout mice. IGF1 receptor (IGF1R) phosphorylation was also increased in *Ndufs4*^{-/-} mice and reduced by rapamycin (Fig. 4A). Whole-body quantitative magnetic resonance revealed a progressive loss in body fat in the *Ndufs4*^{-/-} mice that was ameliorated by daily rapamycin injections (Fig. 4B). Furthermore, Oil Red O staining and metabolomic analysis of liver indicated that knockout mice had a marked deficiency in liver fat droplets and free fatty acids that was partially rescued by rapamycin (Fig. 4, C and D, and fig. S13). Whole-brain metabolomics of 30-day-old mice revealed an abnormal metabolic profile in the *Ndufs4*^{-/-} mice that included an accumulation of pyruvate, lactate, and all detected glycolytic intermediates, consistent with clinical reports of Leigh syndrome (2, 15) (Fig. 4E and table S1). The metabolomic signature of the *Ndufs4*^{-/-} mouse brain includes a decrease in free amino acids, free fatty acids, nucleotides, and products of nucleotide catabolism, increased oxidative stress markers, and reduced levels of γ -aminobutyric acid (GABA) and dopamine (fig. S14 and table S1). Rapamycin rescued many of these metabolomic defects associated with NDUFS4 deficiency, including levels of GABA, dopamine, and free fatty acids. Increased amino acids, metabolites of amino acid and nucleotide catabolism, and free fatty acids accompanied the decrease in glycolytic intermedi-

ates, whereas markers of oxidative stress were unchanged. Moreover, hexokinase—the first enzyme in glycolysis—was increased in *Ndufs4*^{-/-} mice and reduced by rapamycin in knockout and control mice, consistent with the decrease in glycolytic intermediates (Fig. 4A).

Taken together, our results demonstrate that inhibition of mTOR improves survival and health in the *Ndufs4*^{-/-} model of Leigh syndrome. These findings raise the possibility that mTOR inhibitors may offer therapeutic benefit to patients with Leigh syndrome and potentially other mitochondrial disorders. Rapamycin derivatives have several adverse effects, however, including immunosuppression, hyperlipidemia, and decreased wound healing, which may limit their utility in this context, particularly in very young patients. Thus, a more detailed understanding of the mechanisms by which rapamycin alleviates disease in *Ndufs4*^{-/-} mice should allow for the development of more targeted interventions to improve health in patients suffering from mitochondrial diseases for which there are no effective treatments.

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Supplementary Materials

www.sciencemag.org/content/342/6165/1524/suppl/DC1
Materials and Methods
Figs. S1 to S14
Tables S1 to S3

7 August 2013; accepted 6 November 2013
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PHOTOSYNTHESIS RESEARCH at Okayama University

If it weren't for a tiny cluster of manganese, calcium, and oxygen atoms found inside the chloroplasts of plant cells, Earth would be a very different planet. Those atoms form the catalyst that allows plants to split water molecules using sunlight—the first step in photosynthesis. Without them, plants and other photosynthetic organisms wouldn't be able to use solar energy to grow, and they wouldn't give off the oxygen we breathe. Yet exactly how that key catalyst works is still a mystery.

Researchers at Okayama University in central Japan are leading the search for an answer. Two years ago, they published the most detailed images ever seen of the catalyst and the protein that carries it, drawing international attention. Now they are working to capture images of the water-splitting process in action. Their findings could help revolutionize the way we power human civilization.

“Right now we depend on fossil fuels for energy, but in dozens or hundreds of years those will eventually be used up and we will have to depend on the energy of sunlight,” says Jian-Ren Shen, a professor of molecular biophysics at the university and head of a new photosynthesis research center there. “If we can understand the mechanistic basis of this reaction, then we may be able to synthesize an artificial catalyst to split water into protons and electrons. These can then be recombined to generate hydrogen gas.”

The idea is to store the sun's power not in the bonds of sugar molecules, like plants do, but in fuels that humans can use to drive cars or power factories. Hydrogen is one option; methanol, produced by combining hydrogen with carbon dioxide and oxygen, is another. Both fuels are easy to store and transport, which gives “artificial photosynthesis”—and the solar fuels it generates—an advantage over solar electricity.

Scientists have already developed a number of artificial photosynthetic systems that work in the laboratory. So far, however, no artificial system converts solar energy to chemical energy



Dean of the Graduate School of Natural Science and Technology, Professor Shen (center), with President of Okayama University, Kiyoshi Morita (right)

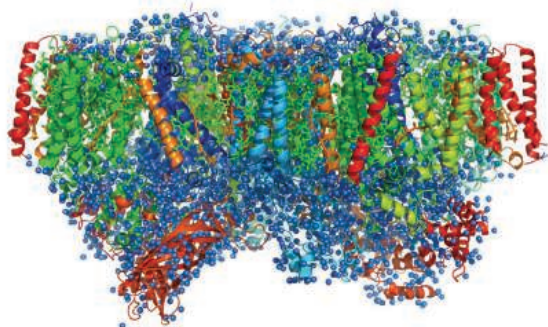
as efficiently as plants do. (Seventy to eighty percent of the solar energy captured by chloroplasts is retained through the first step of photosynthesis, although only between 0.1 and 3 percent typically makes it to the final step of conversion to sugars.) Another problem is that the materials in many artificial catalysts are neither cheap nor abundant. Plants could inspire better alternatives. “It may be difficult to exactly mimic the natural systems, but by understanding their principals, we can make something similar to them,” Dr. Shen says.

His investigation of those principals began over 20 years ago. At the time, he was studying how atmospheric pollutants affect plant growth. He noticed that pollution lowered photosynthetic activity, but when he tried to figure out exactly which step was inhibited, he encountered a problem: scientists didn't fully understand photosynthesis itself. Dr. Shen decided to shift the focus of his research to clarifying the first step in the complex chain of reactions, during which photons from sunlight enter a protein called photosystem II (PSII) and split water molecules into oxygen, hydrogen ions, and electrons. Because the bonds in water molecules are too strong to be broken by sunlight alone, the reaction requires a catalyst to lower the amount of energy that's needed. Dr. Shen wanted to identify and characterize that catalyst.

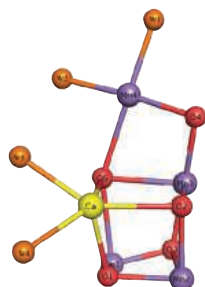
The goal would prove a difficult one to achieve. PSII is a complex of 20 different subunits, some of which are bound together only weakly; the proteins Dr. Shen was working with contained more than 50,000 atoms each (excluding hydrogen). These had to be extracted intact from cells and then purified—a “major difficulty,” he says. He began experimenting with a number of combinations of detergents, salts, and pH levels to figure out which one dissolved the substances surrounding PSII most effectively without harming the protein itself.

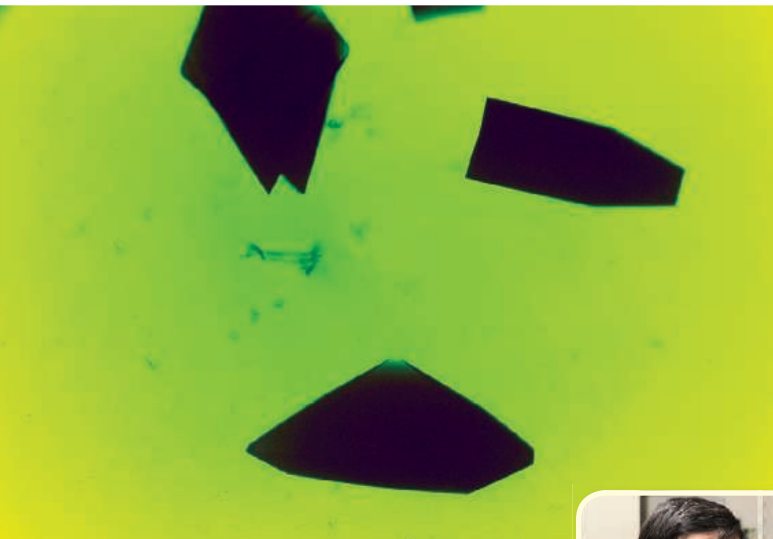
At the same time, he began a series of experiments to find the best conditions for turning the solution of proteins into high-quality crystals.

Protein Structure of Photosystem II



Catalyst for Water Splitting





Crystals of Photosystem II

Electromagnetic radiation was fired at the crystals to reveal the arrangement of atoms within them, a process called X-ray crystallography. But the larger the protein, the more imperfections its crystals tend to have, and the lower the resolution of images. PSII is enormous with a molecular weight of around 700 kDa. It took Dr. Shen and his colleagues 13 years to grow crystals that yielded images at a resolution of 3.7 Å. However, the bonds separating atoms in PSII's catalytic center are as short as 1.8 Å, so Dr. Shen still couldn't decipher its structure.

In 2003, he joined the faculty at Okayama University and continued his experiments there. The school was an ideal place for him to do the work, with its history of pioneering photosynthesis research reaching back to the 1960s. But in the United Kingdom and Germany, research teams were also homing in on PSII's catalytic center. By 2009, German scientists had captured images of the protein at a resolution of 2.9 Å.

That same summer Dr. Shen and his graduate students grew a batch of crystals that seemed promising. They brought them to SPring-8, one of the world's top synchrotron facilities for X-ray crystallography, located just an hour and a half from Okayama University. There they gathered data on the protein's structure in collaboration with members of a team led by Nobuo Kamiya, a professor of structural biology at Osaka City University. Dr. Kamiya took the data back to his lab for analysis.

What came out of the data was the most detailed map of PSII ever seen, making PSII the largest membrane-protein complex with its structure solved at an atomic resolution. At a resolution of 1.9 Å, the exact arrangement and number of atoms in the catalytic core became clearly visible. One calcium, four manganese, and five oxygen atoms were arranged in what Dr. Shen describes as a "distorted chair" shape surrounded by four water molecules.

One back corner of the "seat" appeared slightly raised, which meant the bonds between it and the atoms adjacent to it were longer—and probably weaker—than other bonds in the structure. "These bonds can move and even be easily cleaved," Dr. Shen explains. He suspects this flexibility is important during the catalytic reaction.

Drs. Shen and Kamiya published their findings in *Nature* in 2011, to international acclaim. *Science* magazine named the research one of the top ten breakthroughs of the year, and in 2012 they won a prestigious Asahi Prize for their work. But by then Dr. Shen was already focused on the next step forward: investigating how the PSII catalyst functions during the water-splitting reaction. So far, he has a snapshot of the opening scene. He wants a feature film. His strategy is to illuminate the protein with a single photon, allow the reaction to reach an intermediate stage, and then quickly lower the temperature to freeze the catalyst in action for imaging.

Other researchers around the world are racing to do the same thing. Dr. Shen's group, however, has the advantage of excellent crystals as well as the use of a new imaging facility called SACLA that opened within the campus of SPring-8 in March 2012. SACLA is an X-ray Free Electron Laser that can reveal the movement of atoms and molecules in real time—something even the extremely powerful SPring-8 X-ray beams cannot do.

That technology will be key to achieving Dr. Shen's current research goal.

Okayama University has thrown its full support behind the groundbreaking research. In April of 2013, the school established a new Photosynthesis Research Center focused on three core projects, one of which belongs to Dr. Shen. The second project, led by molecular biologist Yuichiro Takahashi, is looking at how environmental stresses like drought or excessive sunlight impact photosynthesis, work that holds important implications as climate change begins to affect agricultural productivity. The center's third project is led by inorganic chemist Takayoshi Suzuki, who is applying Dr. Shen's findings to research into synthetic compounds that split water with sunlight. Together, these projects promise not only to keep Okayama University at the forefront of fundamental research, but also to advance the practical applications that are key to a sustainable future.



Professor Shen in the Lab



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Dr. Graetzel is a professor and director of the Laboratory of Photonics and Interfaces at the École polytechnique fédérale de Lausanne. In spring 2014, he will give a public talk in Louisville, KY on his winning work and receive the \$50,000 Conn Prize and medal, which recognizes outstanding renewable energy ideas and achievements with proven global impact.

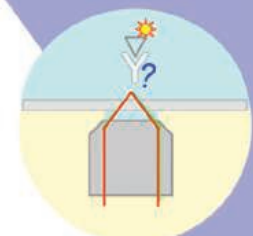
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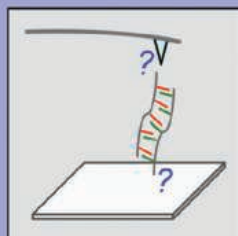
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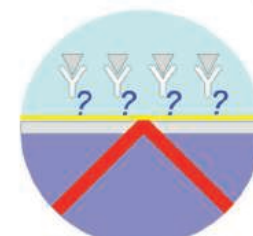
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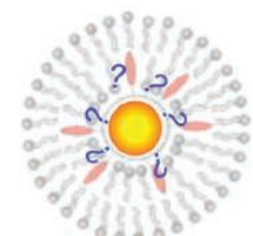
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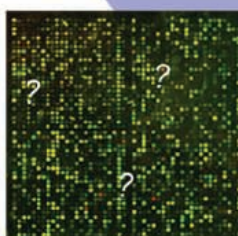
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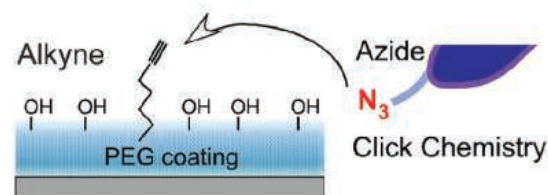
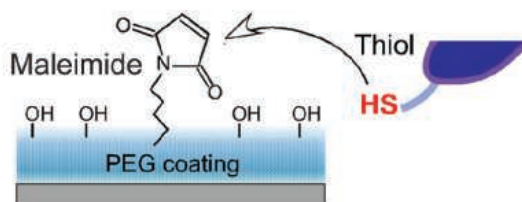
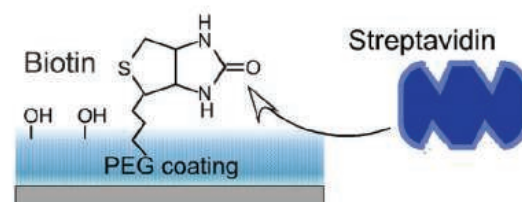
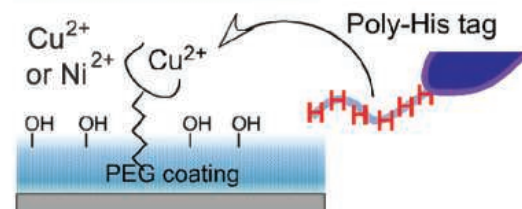
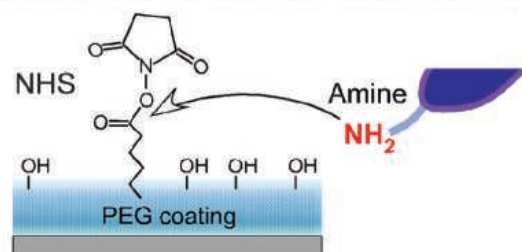
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MY-PCR Prep provides the molecular biology laboratory technician with a "personal cleanroom" for use in the amplification of DNA and RNA. Between amplifications, My-PCR Prep can be irradiated with shortwave ultraviolet (UV) energy to denature potential contaminants and eliminate their ability to be amplified. The main chamber of MY-PCR Prep is constructed from a continuous piece of polycarbonate to prevent UV light from escaping the chamber during irradiation. Operator access is gained through the folding front polycarbonate sash, overlapped to eliminate gaps in the chamber during UV light irradiation. The work surface is white polypropylene making disinfection and cleaning simple. My-PCR Prep is a Class 100 vertical laminar flow workstation with timed UV light, making it an ideal benchtop personal clean zone for completing DNA and RNA amplifications. My-PCR Prep is available in 24"- and 32"-wide models; 110V AC.

Mystaire Misonix

For info: 919-229-8511 | www.mystaire.com

CALCIUM KIT

The Fura-2 QBT Calcium Kit combines the calcium indicator Fura-2 with Molecular Devices' proprietary masking technology to provide a fast, simple, and reliable ratiometric fluorescence-based assay for detecting and quantitating changes in intracellular calcium. Utilizing Molecular Devices' masking technology, the Fura-2 QBT Calcium Kit significantly reduces background fluorescence in media around the cells, enabling researchers to more easily detect signal from within the cell, giving more accurate results. Moreover, the new kit eliminates the need for wash protocols, thus saving time and further improving data quality. Leveraging the advantages of Fura-2 over other calcium indicators, the dye is excited at two different wavelengths, enabling ratiometric measurement of calcium flux to minimize the impact of assay variances caused by uneven dye loading, leakage, or cell density. The Fura-2 QBT Calcium Kit enables detection of the largest signal window available for Fura-2, and allows scientists to interrogate low-density, weakly, or nonadherent cells.

Molecular Devices

For info: 800-635-5577 | www.moleculardevices.com

SEQUENCING KITS

New reagent kits are now available for the MiSeq, the industry's most accurate and easiest-to-use benchtop sequencer. The improved chemistry doubles sequencing output to 15 gigabases (Gb) by increasing the number of sequencing reads (up to 25 million reads) and overall read length (up to 2x300 base pairs). These innovations enable researchers to perform new applications including exome sequencing on the MiSeq. In addition, increased sequencing reads will support transcriptome applications such as mRNA sequencing and will offer higher throughput capacity for gene expression profiling with Illumina's TruSeq Targeted RNA Expression assay. The benefits of increased paired-end read lengths include improving the quality of genome assemblies and advancing applications that require longer read lengths, such as metagenomics and human leukocyte antigen (HLA) typing.

Illumina

For info: 800-809-4566 | www.illumina.com/miseq

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Join Keystone Symposia for Two Unique Events

HIV/AIDS: Strategies for an Endgame

December 13, 2013 | 1:00–2:30 PM EST

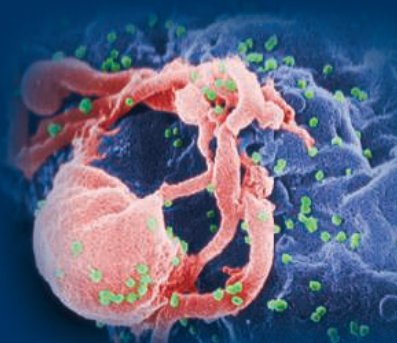
Our first FREE, LIVE webcast.

This 90-minute event will aim to determine the optimal strategy to end the AIDS epidemic, with a particular focus on discussing the merits of pre-exposure prophylaxis versus vaccines.

Moderator: Bruce Walker

Panelists: Myron (Mike) Cohen, Betsy Herold, Julie McElrath and Gary Nabel

Visit www.keystonesymposia.org/KSHIV to register and to submit a question/topic that you would like addressed during the discussion.



Big Data in Biology

March 23–25, 2014 | San Francisco, California, USA

Our first SHORT, TWO-DAY symposium.

This conference will address the challenges of sharing, archiving, integrating and analyzing the vast amounts of biological data now being generated. The event will bring together various research specialties that rarely interact, including plant scientists, medical geneticists, genomicists, microscopists and neurobiologists, as well as computer scientists, computational biologists, mathematicians and technologists. Event sessions start at 8 AM on March 24.

Scientific Organizers: Lincoln D. Stein,
Doreen Ware and Michael Schatz

Session Topics:

- Databases and Clouds
- Panel on Big Data Challenges and Solutions:
Control Access to Individual Genomes
- Personal Genomes
- Imaging/Pharmacogenomics

Discounted Abstract Deadline: **November 19, 2013**

Student/Postdoc Scholarship Application Deadline: **November 19, 2013**

Abstract Deadline: **December 18, 2013**

Discounted Registration Deadline: **January 21, 2014**

For more information and to view the full program,
visit www.keystonesymposia.org/14F2

CONFIRMED SPEAKERS

(as of November 4, 2013):

Laura Clarke, European
Bioinformatics Institute
Mark Gerstein, Yale University
David Haussler*, UC Santa Cruz
Jill P. Mesirov, Broad Institute
John Overington, European
Molecular Biology Laboratory
Ajay Royyuru, IBM T.J. Watson
Research Center
Michael Schatz, Cold Spring
Harbor Laboratory
Dan Stanzione, University
of Texas at Austin
Lincoln D. Stein, Ontario
Institute for Cancer Research
Susan Sunkin, Allen Institute
for Brain Science
Jason Swedlow, University
of Dundee
Matt Wood, Amazon Web
Services, Inc.

**Keynote Speaker*



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Science Careers

From the journal *Science*



ScienceCareers.org

Medical University of South Carolina

Department of Pediatrics

Recruitment for Endowed Professorship in Center for Childhood Neurotherapeutics, Basic Research in Childhood Neurological Disorders



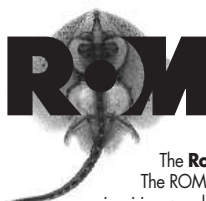
The Department of Pediatrics and the Medical University South Carolina at Charleston is accepting applications for an Endowed Chair for Basic Research in Childhood Neurological Disorders at the rank of a Professor supported by SmartState Award from the Center of Economic Excellence (COEE), State of South Carolina. We are seeking an outstanding basic research scientist with expertise in disease mechanisms and therapeutic approaches for childhood diseases of the nervous system who would complement and expand existing programs at MUSC. Applicant should have a terminal degree (Ph.D., M.D. /Ph.D. or M.D.) with appropriate research expertise in neurosciences. Candidate should have a national reputation, outstanding track record of high quality publications and a strong record of extramural funded research.

Endowed Chair will have the benefit of a significant programmatic and departmental startup package and laboratory space in the newly built Darby Children's Research Institute (DCRI) housed with state of art shared Lipidomics and Proteomics core facilities are available. The interdepartmental children's research program supports a highly collaborative environment with opportunities for both basic and translational research.

MUSC is located in Charleston, SC, a city noted for its historical, cultural and recreational attractions with a temperate climate and beautiful beaches. Charleston is consistently given top honors as a most desirable place to live. Charleston's Academic Magnet High School was ranked the #1 magnet high school in the State and #10 in the country by US News and World Report.

Interested scientists should submit a CV, summary of research interests, and contact information for three references to **Inderjit Singh, PhD**, Chief, Division of Developmental Neurogenetics, (singhi@musc.edu), or to **Rita M. Ryan, MD**, Chair, Department of Pediatrics, (ryanr@musc.edu).

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The **Royal Ontario Museum (ROM)** connects visitors to their world and each other. The ROM is an indispensable resource for building community by nurturing discovery and inspiring wonder. The ROM invites everyone to explore and enjoy extraordinary experiences of science and civilization.

Invertebrate Zoologist

The ROM houses some of Canada's most important collections in both Natural History and World Cultures. The ROM invites applications for the position of an entry-level (equivalent to Assistant Professor) **Curator of Invertebrate Zoology** (exclusive of terrestrial arthropods) to conduct field and collections-related research.

The successful applicant will be expected to: develop a program of externally funded scholarly research and publications; curate and continue building the disciplinary collection of invertebrates; participate in the development and rotation of new permanent galleries, and travelling exhibitions; and actively participate in, and contribute to, the development of public programming.

Applicants will have a PhD in Systematic Biology and be well versed in phylogenetic methods for analyzing DNA and/or morphological datasets, phylogenomics, evolutionary biology or historical biogeography; have a record of scholarly publication in peer-reviewed journals; be qualified for cross-appointment to the University of Toronto and be eligible for NSERC funding in support of research (i.e., proven record of successful grant applications). Experience in a museum or equivalent environment is preferable.

Salary and rank are commensurate with experience as stipulated in the Collective Agreement between the ROM and the ROM Curatorial Association. Applicants should provide a curriculum vitae, a summary of their research, and an outline of their proposed research, and should arrange to have three confidential letters of recommendation sent on their behalf, to: **Human Resources Department, The Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada, M5S 2C6. Fax: 416-586-5827.** Applications for the position will be accepted until **January 15, 2014**. All qualified candidates are encouraged to apply; however, Canadians and permanent residents of Canada will be given priority.

The ROM is committed to fair and accessible employment practices. Upon request, suitable accommodations are available under the Accessibility for Ontarians with Disabilities Act (AODA) to applicants invited to an interview.



www.rom.on.ca

Faculty Positions



Mammalian Genetics, Bar Harbor, Maine

The Jackson Laboratory for Mammalian Genetics, in Bar Harbor, Maine, is inviting applications for **Assistant, Associate and Full Professors**. Faculty on the Bar Harbor campus use genetic and genomic approaches to conduct research on fundamental biological problems and mechanisms of disease using the mouse as a model organism. Faculty members are being recruited for research in diverse areas, including:

- Cancer Biology
- Computational Biology
- Neuroscience
- Developmental and Reproductive Biology
- Aging
- Immunology
- Transgenerational Inheritance and Epigenetics
- Statistical and Systems Genetics
- Genomic Editing and Engineering

Candidates must have a Ph.D., M.D., or D.V.M., and relevant postdoctoral training with an exceptional record of research accomplishment, and the ability to develop a competitive, independently funded research program. Opportunities are available for shared mentorship of trainees, and integration with The Jackson Laboratory campuses in Connecticut and California.

The Jackson Laboratory offers a uniquely collaborative scientific research environment. Faculty are supported by outstanding scientific services, unparalleled mouse and genomic resources, postdoctoral and predoctoral training programs, and numerous courses and conferences centered on the mouse as a genetic model for human biology and disease.

Applicants must apply online. Please submit a curriculum vitae and a concise statement of research interests as one document to www.jax.org/careers, click on Faculty Positions. In addition, please have three letters of reference sent to: facultyjobs@jax.org. The application deadline is February 15, 2014.

For inquiries, please contact Dr. Robert Braun, Professor and Vice President of Research of The Jackson Laboratory, at bob.braun@jax.org. Information about The Jackson Laboratory for Mammalian Genetics and its current faculty may be found at <http://www.jax.org>.

Genomic Medicine, Farmington, Connecticut

The Jackson Laboratory for Genomic Medicine in Farmington, Connecticut is inviting applications for **Assistant, Associate and Full Professors**. The campus is dedicated to advancing precision medicine using genetic and genomic strategies to understand the complex functional networks underlying health and disease, and the development of novel diagnostics and therapeutics. We are seeking individuals to join our interactive culture of cooperation and program integration. Areas for recruitment include:

- Computational Biology and Bioinformatics
- Functional Genomics and Genomic Technologies
- Systems Biology
- Human Genetics and Morbid Genetics
- Statistical Genetics and Population Genetics
- Cancer Biology and Cancer Genomics
- Immunology and Infectious Diseases
- Neurology and Neurobiology
- Metabolic Disorders
- Genetics of Aging
- Biomarker Development and High Content Screening

Candidates with M.D. and/or Ph.D. degree(s), and relevant postdoctoral training, and with either academic or industry experience will be considered. There will be shared mentorship of trainees, common platforms, and integration with the other Jackson Laboratory campuses in Maine and California.



For inquiries, please contact Dr. Charles Lee, Scientific Director and Professor of The Jackson Laboratory for Genomic Medicine, at charles.lee@jax.org. Information about The Jackson Laboratory for Genomic Medicine and its current faculty may be found at <http://www.jax.org/ct/>.

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Icahn School of Medicine at Mount Sinai is one of the world's leading biomedical institutions and internationally acclaimed for excellence in scientific research, clinical care and education. It is among the nation's top twenty medical schools in NIH funding and U.S. News and World Report rankings. The School offers education programs leading to M.D., Ph.D. and master's degrees, and attracts outstanding students to its highly competitive programs and invigorating academic environment.



**Icahn
School of
Medicine at
Mount
Sinai**

SENIOR FACULTY POSITION IN BIOLOGICAL MASS SPECTROMETRY FOR NEUROSCIENCE OR CANCER BIOLOGY RESEARCH

Icahn School of Medicine at Mount Sinai is seeking a qualified candidate for a senior faculty position in proteomics-based research in the areas of neuroscience or cancer biology. We invite applications from established investigators applying mass spectrometry techniques to understand basic disease-related mechanisms in the areas of neuroscience or cancer biology.

Appointments are available at the level of Associate or Full Professor. Individuals must have a Ph.D. or equivalent degree in a relevant research field and applicants with current research funding will be given preference. The ideal candidate uses quantitative mass spectrometry tools and has expertise in characterizing posttranslational modifications in proteins.

Positions carry highly competitive salary and start-up packages. Interested candidates should submit their Curriculum Vitae, a brief description of their research accomplishments and proposed research program. Complete contact information for at least three professional references should also be provided. Materials should be sent as a single PDF file, to: proteomicssearch@mssm.edu

Mount Sinai Health System is an equal opportunity/affirmative action employer. We recognize the power and importance of a diverse employee population and strongly encourage applicants with various experiences and backgrounds.

FACULTY POSITIONS AT TIANJIN UNIVERSITY 天津大学

Founded in 1895 as Peiyang University, Tianjin University (TU) is regarded as the pioneer of modern higher education in China. Supported by Chinese Ministry of Education, TU is among the first group of universities to be included in the "985", "211" and "2011" Projects of national investment for developing world recognized universities. Over the years, TU has grown into a world prestigious research university with distinctive quality and strength in education, research and social services. (www.tju.edu.cn)

Positions: TU invites outstanding applicants for full-time professorship, and looks for candidates in the following areas. **In Science or Engineering:** Engineering, natural science, life science, information technology, relevant emerging inter-disciplines, etc. **In Other Academic Fields:** Architecture, economics, business, management, social sciences, relevant emerging inter-disciplines, etc. Meanwhile, applicants with research background of multi-disciplinary and non-traditional approach are highly expected.

Qualifications: Competitive applicants with outstanding academic performance shall be academic staff from leading university, scientists and researchers from renowned institute and company. Research excellence and potential for future productivity are essential. Additional criteria include leadership and communication skills. **In Science or Engineering:** Applicants shall apply for National Recruitment Program of Global Youth Experts (the National Youth 1000-Talent Program) through TU. Successful candidates will be deemed as the appointee. **In Other Academic Fields:** Applicants will be selected according to their qualifications, academic performance, innovation capability, and leadership.

Responsibilities: Responsibilities include establishing a vigorous research program, teaching undergraduate and graduate students, and providing professional/institutional services.

Salary and Support: TU offers an attractive remuneration package. Salary will be commensurate with candidates' qualifications, academic performance and experience. In addition, TU start-up package provides research grant, lab/office space and research-team support.

In Science or Engineering:

- An annual pre-tax salary ranging from 400K to 600K RMB will be offered to appointee.
- An annual pre-tax salary ranging from 350K to 400K RMB will be offered to the candidates who are shortlisted for interview but not selected in the National Youth 1000-Talent Program.

In Other Academic Fields:

- Salary is offered by referring to that of candidates in Science or Engineering.

Application Procedure: Please submit electronically a complete application package consisting of the following documents to oplan@tju.edu.cn. The application deadline is **March 15, 2014**.

(1) Application form, (2) Detailed curriculum vitae, (3) Publications listing and five full-text representative publications. As for the detailed application procedure and application form, please download from <http://hr.tju.edu.cn/zpxx/js/>

Contacts: Contact Persons: Dr. LIU Na, Ms. ZHANG Yinlu, Human Resource Department, Tianjin University, China, **E-mail:** oplan@tju.edu.cn, **Telephone:** (+) 86-022-27403932, (+) 86-022-27402079 **Fax:** (+) 86-022-27404177 **Address:** 223/Building 9, 92 Weijin Road, Nankai District, Tianjin, 300072



FULL TIME FACULTY POSITIONS AT UNIVERSITY OF SCIENCE AND TECHNOLOGY OF CHINA (USTC)

The University of Science and Technology of China (USTC), is one of the most prestigious universities in China and the only university jointly endowed by both the Chinese Academy of Sciences (CAS) and the Ministry of Education of China (MOE). Widely recognized for its culture and tradition in talent focus and academic priority, USTC is a thriving institution with stimulating atmosphere for ground-breaking research and innovation. USTC scholars enjoy enormous freedom in realizing their own research aspirations and gaining international visibility, and USTC students are reputed in their deep fond of science and independent thinking. Numerous USTC alumni have become world-renowned scientists and entrepreneurs.

POSITIONS

National Recruitment Programs			
POSITION		QUALIFICATIONS	
1000 Talents Program (1000Plan) Professorship for Young Scholars		• <40 yrs old • Overseas PhD degree + no less than 3 consecutive years of overseas postdoc research experience • Or Chinese domestic PhD degree + no less than 5 consecutive years of overseas postdoc research experience • Demonstrated excellence in publication & experience in internationally visible research projects	
100 Talents Program of Chinese Academy of Science (CAS 100Pan) Professorship		• <45 yrs old • PhD degree + No less than 4 consecutive years of postdoctoral overseas research experience • Demonstrated excellence in publication & experience in internationally visible research projects	
Chang Jiang Scholars Program of Chinese Ministry of Education -Distinguished Professorship		• <45 yrs old • Currently an associate professor, or in an equivalent position, of a world renowned research institution • Proven excellence in research achievements	
Tenured Positions		Contract Positions	
POSITION	QUALIFICATIONS	POSITION	QUALIFICATIONS
• Full Professor • Associate Professor	• Demonstrated excellence in publication, funding or patent record • Strong experience in establishing research programs • Academic rank will be commensurate with experience	• Research Professor • Research Associate Professor • Postdoctoral Researcher	• Upon specific needs of each research group (Please visit USTC website at http://en.ustc.edu.cn, and explore the hiring information of each school)

SUPPORT, SALARY AND BENEFITS

USTC is featured with and proud of its highly collegial, interactive, and supportive environment for researchers. Besides, it offers competitive start-up package among all the "C9 League" universities of China. The benefits include spacious housing, subsidized faculty dining, premium medical services, etc. Professors hired through the recruitment programs will receive supplementary remuneration, as well as assistance on the establishment of a dedicated research team. The start-up package includes adequate start-up funds, newly renovated office, ample laboratory space, and plenty of research assistants. Please visit the USTC Talent Recruitment and Services website for more details: <http://employment.ustc.edu.cn>.

ACADEMIC DISCIPLINES

USTC has a broad range of academic disciplines, covering natural sciences, engineering, management, and some emerging interdisciplinary fields. All disciplines are open for employment opportunities, with particular emphasis on enhancing and broadening USTC's strengths and representation in engineering and high tech areas, such as nuclear science & engineering, future internet, advanced manufacturing, etc.. The University embraces applicants of all ranks, from internationally established investigators who would establish their own research groups and platforms, to junior scholars who may complement our existing research programs.

CONTACT US

Qualified applicants are invited to send a job intention letter and a curriculum vitae to: job@ustc.edu.cn.

Mailing Address:

Talents Recruitment & Services Office
University of Science and Technology of China
96 Jinzhai Road, Hefei, 230026, P.R. China

Telephone: +86-551-63607769

Fax: +86-551-63637049



Stony Brook University

Genomics Faculty Cluster Hire

As part of a new initiative in Genomics, Stony Brook University invites applications for six tenure-track faculty positions in the broad areas of genomics, sequencing, and informatics.

We are seeking candidates with interest and expertise in using high-throughput sequencing and other modern genomics tools to answer questions related to the ecology and the physiology of living organisms, as well as candidates interested in developing new methods and approaches to generate and analyze genomics data. Positions may be filled in coordinated sets; e.g., the simultaneous hire of faculty with expertise in experimental genomics/sequencing and faculty with expertise in informatics. In addition to colleagues and resources at Stony Brook and its medical school, cluster faculty will have access to the resources of the New York Genome Center (nygenome.org), of which Stony Brook University is a Founding Member. Successful candidates would be expected to develop an internationally recognized research program, supervise student research and teach courses related to their field. Academic appointments for cluster faculty will be based on their areas of research and will be in the School of Marine and Atmospheric Sciences, as well as in the departments of Ecology and Evolution, Molecular Genetics & Microbiology, Biomedical Engineering and Computer Science. Further description of each position is available at www.stonybrook.edu/clusterhires

Required Qualifications: Doctoral degree in appropriate discipline; demonstrated outstanding research strength; a strong disciplinary background; interest in interdisciplinary research; and strong experimental and/or theoretical skills.

Preferred Qualifications: Strong publication record in discipline. Graduate and/or undergraduate teaching experience.

Special Notes: These are tenure track positions. FLSA Exempt position, not eligible for the overtime provisions of the FLSA. It is expected that the positions will be filled at the Assistant Professor level; however, exceptional candidates at higher ranks will also be considered. Application review will begin in January 2014 and will continue until all positions are filled. For full descriptions of the positions and application instructions, visit www.stonybrook.edu/clusterhires.

To view application procedure, full position descriptions, or to apply online, visit www.stonybrook.edu/jobs (Ref. # F-8315-13-11).

Stony Brook University/SUNY is an equal opportunity, affirmative action employer.



The University of California at Davis is pleased to announce recruitment for a tenure-track faculty position in Applied Animal Behavior. The successful candidate will join the Department of Animal Science in the College of Agricultural and Environmental Sciences at the rank of Assistant Professor. Criteria for appointment include: a Ph.D. or equivalent in animal behavior or a closely related field, a strong interest in animal behavior of domestic animals relevant to animal agriculture, a record of excellence in scholarly research, and demonstrable potential to establish a competitively-funded research program relevant to animal behavior. The appointee will be responsible for teaching undergraduate courses in animal ethics and animal welfare, be actively involved in undergraduate advising, curricular development and department and university service. The appointee is also expected to guide and mentor graduate students and participate in research and outreach programs consistent with the mission of the CA Agricultural Experiment Station.

Applicants should apply via the following website: <https://recruit.ucdavis.edu/apply/JPF00204>. The position will remain open until filled but to ensure consideration, applications should be received by **March 15, 2014**.

UC Davis is an Affirmative Action/Equal Employment Opportunity Employer and is dedicated to recruiting a diverse faculty community. We welcome all qualified applicants to apply, including women, minorities, veterans, and individuals with disabilities.

Northeastern University

College of Engineering



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53 ENGINEERING FACULTY HIRES SINCE 2010...and counting

Northeastern's College of Engineering seeks outstanding faculty candidates for appointments at the associate or full professor level, both within the college and in conjunction with interdisciplinary positions across the university. We will also consider exceptional candidates at the assistant professor level. Successful applicants will lead internationally recognized research programs that complement existing expertise aligned with one or more of Northeastern's strategic research themes—health, security, and sustainability—or in the enabling fields of nanotechnology and materials, and data science. Our Boston campus facilitates collaborations with major medical centers, research labs, neighboring academic institutions, and industry.

Northeastern is an Affirmative Action/Equal Opportunity Educator and Employer committed to excellence through diversity.



The OECD's mission – Better Policies for Better Lives – promotes policies that will improve the economic and social well-being of people around the world. It provides a unique forum in which governments work together to share experiences on what drives economic, social and environmental change, seeking solutions to common problems.

This is a Paris-based position with an attractive remuneration package.

Senior Policy Analyst Directorate for Science, Technology and Industry www.oecd.org/sti

The Science, Technology and Industry Directorate (STI), which employs about 130 staff, contributes to the Organisation's mission in assisting member and key economies, including emerging economies, build an environment conducive to translating science, technology and knowledge into innovation in order to improve economic performance and enhance social welfare.

We are looking for a Senior Science Policy Analyst to manage and contribute to all aspects of the OECD's work on issues at the intersection of science and public policies, carried out through the OECD Global Science Forum (GSF) under the auspices of its parent committee, the Committee for Scientific and Technological Policy.

The GSF provides a venue for consultations among senior science policy officials of OECD member countries and key non-member economies on high-priority science policy issues requiring international consultations/co-operation. It produces findings and action recommendations on such policy issues and identifies opportunities for collaboration on major scientific undertakings.

The successful candidate will have extensive experience in providing advice on science policy issues through work at senior level in a national administration or international organisation, as well as an excellent understanding of the range of key scientific, technological, economic and regulatory issues relevant to the GSF. S/he will lead and motivate a small team.

This position requires fluency in one of the two OECD official languages (English or French) and a good working knowledge of the other.

Full details of the position are available at www.oecd.org/careers/vacancies/ref09009. Applications from OECD member country nationals should be submitted online by **10 January 2014**.



Faculty Positions Chemical Sciences

The Physical Sciences and Engineering (PSE) Division at King Abdullah University of Science and Technology (KAUST) invites qualified applicants to apply for faculty positions at all ranks (Assistant, Associate, and Full Professor) in the Chemical Sciences Program.

KAUST offers superb research facilities, generous assured research funding and internationally competitive salaries. www.kaust.edu.sa

The science produced in PSE is about understanding, modeling, and manipulating matter at all scales: nano, meso, and macroscopic levels; in all forms: bulk, thin films, divided colloids, fluid flows, earth as system etc. and in interaction with external stimuli: light, heat, fluids, etc. or stresses. The knowledge created serves to design and engineer materials, technologies, and systems.

The Chemical Sciences Program is concerned with Chemistry in all its facets including those addressed in KAUST Research Centers, particularly in Catalysis, Membrane, Solar Energy, and Red Sea Centers. <http://chems.kaust.edu.sa>

The Chemical Sciences program is currently recruiting for the following positions:

POLYMERIC MATERIALS

- Design, synthesis, and structural/ molecular characterization
- Investigation of functional properties of novel well-defined polymeric materials

EXPERIMENTAL POLYMER PHYSICS

- Emphasis on dynamics and molecular rheology of polymeric systems including, but not limited to, branched polymers, copolymers, functionalized and responsive polymers, nanocomposites, melts and solutions
- Particular interest in applicants with research experience and related background in polymer processing

TOTAL SYNTHESIS (IN COLLABORATION WITH THE RED SEA AND PLANT CENTERS)

- Initiate organic chemistry research program dedicated to total synthesis of biologically active natural products

HETEROGENEOUS CATALYSIS (POSITION OPEN IN KAUST CATALYSIS CENTER: [HTTP://CCRC.KAUST.EDU.SA](http://ccrc.kaust.edu.sa))

- Focus involves "New Concepts in Catalysis leading to Major Breakthroughs"
- Expand the heterogeneous catalysis expertise to the area of refining and petrochemistry
- Exploit the unique situation of Saudi Arabia which houses almost one third of oil reserves in the world

Applicants should have a proven track record to establish a high impact research program and have a commitment to high quality teaching at the graduate level.

To learn more about the PSE Division and complete the online application form, visit <http://apptrkr.com/411370>. Application requirements include the following sections:

- Updated curriculum vitae with a full list of publications
- Statement of research
- Statement of teaching interests
- Contact details of at least four potential referees

Applications received by **January 31, 2014** will receive full consideration and positions will remain open until filled

www.kaust.edu.sa





Biology Full-Time Tenure Track Faculty Position in Developmental Biology

The Boston College Biology Department invites applications for a full-time, tenure-track faculty appointment in Developmental Biology at the **Assistant Professor** or **Associate Professor** level. The Boston College Biology Department is actively expanding and provides competitive start-up funds and research space, with the expectation that the successful candidate will establish, or bring to the University, a vigorous, externally-funded research program. Boston College also provides access to well-equipped animal facilities, core laboratories with state of the art instrumentation for imaging and flow cytometry, and substantial computational resources. The successful applicant should apply cutting edge technologies to a model organism to address major questions in developmental biology. Candidates must possess a Ph.D., have a strong record of continuous research productivity, and have either active funding or clearly demonstrated potential for funding. This appointment will begin on or after July 1, 2014.

Applicants should submit their cover letter, curriculum vitae, a statement of present and future research plans, and arrange to have three letters of reference submitted separately. All application materials can be submitted through Interfolio <http://apply.interfolio.com/24110>.

Applications received before **February 1, 2014** will be given full consideration, but review of applications will continue until the position is filled.

Boston College is an Affirmative Action/Equal Opportunity Employer. To learn more about how BC supports diversity and inclusion throughout the university please visit the Office for Institutional Diversity at <http://www.bc.edu/offices/diversity>.

ASSISTANT PROFESSORS Environmental Studies ARTS AND SCIENCE

Qualified applicants are invited to apply for two full-time, tenure-track assistant professor positions in Environmental Studies, to begin September 1, 2014, pending administrative and budgetary approval. New York University expects to transform the Environmental Studies Program into the Department of Environmental Studies and these positions will be held in that department. We seek excellent scholars with a rigorous theoretical and empirical approach to environmental studies. We are especially interested in candidates with environment-focused expertise in natural resource management, integrative modeling, or urban challenges. Candidates with a Ph.D. in environmental studies or relevant discipline are invited to apply. The successful candidate will be expected to teach, contribute broadly to environmental studies, and play an active role in developing the new department. Candidates should possess a Ph.D. by September 2014, have a research program that demonstrates the potential to be a leader in the field of Environmental Studies, and have proven the ability to be an excellent teacher. The successful candidate will have the opportunity to affiliate with other NYU departments, programs, and centers such as the Marron Institute.

*Application deadline is **February 3, 2014**. To learn more and apply, see the NYU Environmental Studies web site at <http://environment.as.nyu.edu>.*



NEW YORK UNIVERSITY

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Commission

*One Planet, One Ocean
Une Planète, Un Océan*

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UNESCO has the vital mission of initiating dialogue and promoting education among civilisations and cultures to create a peaceful, sustainable future. We believe that only through this dialogue and education can human rights be respected and poverty alleviated.

You will oversee all aspects of strategic operations for the Intergovernmental Oceanographic Commission, with particular emphasis on convening sessions and meetings of the governing and subsidiary bodies. We will look to you to promote and implement agreed programmes and co-ordinate collaboration with international bodies, including United Nations organisations, to build capacity in ocean and coastal area scientific research. In this way, you will be central to meeting international development goals, including those contained in the United Nations Millennium Declaration.

At the same time, you will ensure effective human resource and financial management as well as fundraising, whilst directing the activities of the Secretariat, so you can expect considerable challenge and an extremely high profile. To join us, you will require a postgraduate degree in Ocean Sciences or a related field, backed by extensive experience in relevant senior executive positions, including substantial assignments at regional or international level. This will have given you significant exposure to international co-operation and broad knowledge of UN systems and procedures, and you will be a skilled change manager with the ability to anticipate trends and development in ocean sciences. Finally, you should be an inspiring leader with outstanding strategic and interpersonal skills, strong interest in developing countries, and fluency in one of our official languages of English and French, ideally backed by some command of the other tongue. If needed, we will provide comprehensive language training to sharpen your skills.

To view the full vacancy announcements and apply online, please visit:

<http://en.unesco.org/careers/>

Closing date: January 25, 2014.



ZHEJIANG UNIVERSITY

Faculty positions available in the Department of Physics, Zhejiang University, P. R. China

The Department of Physics at Zhejiang University is expanding its research scope and seeking a number of outstanding candidates to fill in faculty positions at all the professor levels in all subfields of experimental and theoretical physics. Applicants must have a Ph.D. in physics or closely related fields, and a distinguished record of scholarship. The successful applicant is expected to develop an innovative research program and to participate in teaching at the graduate and undergraduate levels. Zhejiang University offers a competitive salary and an attractive benefits package including affordable housing.

Founded in 1897 and located near the West Lake in the beautiful city of Hangzhou, Zhejiang University is one of the top universities in China. With about 60 faculty members, the current research activities of the Physics Department span from various sub-fields including particle physics and field theory, condensed matter physics, atomic, molecular and optical physics, plasma physics, statistical physics and bio-physics.

Further information about the Department of Physics and the faculty members is available at <http://physics.zju.edu.cn/>. Interested candidates should send a copy of *Curriculum Vitae* with a list of publications, five representative papers, and a statement of research interests and plans, and the names and contact information of three references, to Professor Bo Zheng, Chair, Department of Physics, Zhejiang University, 38 Zheda Road, Hangzhou 310027, China, E-mail: zhengbo@zju.edu.cn



Faculty Positions

Chemical and Biological Engineering

The Physical Sciences and Engineering (PSE) Division at King Abdullah University of Science and Technology (KAUST) invites qualified applicants to apply for faculty positions at all ranks (Assistant, Associate, and Full Professor) in the Chemical and Biological Engineering Program.

KAUST offers superb research facilities, generous assured research funding and internationally competitive salaries. www.kaust.edu.sa

The science produced in PSE is about understanding, modeling, and manipulating matter at all scales: nano, meso, and macroscopic levels; in all forms: bulk, thin films, divided colloids, fluid flows, earth as system etc. and in interaction with external stimuli: light, heat, fluids, etc. or stresses. The knowledge created serves to design and engineer materials, technologies, and systems.

The Chemical and Biological Engineering Program offers opportunities to develop real-world solutions to global challenges by leveraging basic discoveries in chemical and biological sciences. The successful candidate will focus his/her research on these required areas of expertise:

PROCESS MODELING AND DESIGN

- Solid academic/industrial background
- Conduct design, optimization, and cost analysis of membrane and conventional separation processes
- Teach advanced principles of process design and control.

PETROLEUM AND NATURAL GAS ENGINEERING (SENIOR-LEVEL POSITION)

- Background and strong knowledge in the petroleum and/or natural gas industry
- Ability to build bridge between KAUST and Saudi Arabia's fast-growing petroleum industry

BIOMOLECULAR ENGINEERING (SENIOR-LEVEL POSITION)

- Development and leadership of Biomolecular Engineering program
- Well-established research in areas such as biomaterials, tissue engineering, bioprocess engineering or biomedical engineering

REACTOR DESIGN AND PROCESS ENGINEERING (POSITION OPEN IN KAUST CATALYSIS CENTER: [HTTP://CCRC.KAUST.EDU.SA](http://ccrc.kaust.edu.sa))

- Specialize in heterogeneous or photo catalysis
- Scale up reactors in field of water splitting, high temperature catalytic processes, and processes for air sensitive catalysts
- Expertise in university, industry, or both

Applicants should have a proven track record to establish a high impact research program and have a commitment to high quality teaching at the graduate level.

To learn more about the PSE Division and complete the online application form, visit <http://apptrkr.com/411352>. Application requirements include the following sections:

- Updated curriculum vitae with a full list of publications
- Statement of research
- Statement of teaching interests
- Contact details of at least four potential referees

Applications received by **January 31, 2014** will receive full consideration and positions will remain open until filled.

www.kaust.edu.sa





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Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

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To learn more, visit
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www.jobs.cam.ac.uk

Herchel Smith Postdoctoral Research Fellowships

Department/Location: Academic Division, Schools of the Biological and Physical Sciences, Cambridge

Salary: £28,132-£36,661

The Managers of the Herchel Smith Postdoctoral Research Fellowships Fund invite applications within the fields of Biological Sciences, Pure Mathematics, Chemistry (organic and/or biochemistry), Condensed Matter Physics, or Materials Science.

All Fellowships are to be held from 1 October 2014 or otherwise by negotiation. Fellowships are available for between two and three years and provide an opportunity for independent research, although the holders will usually work in close collaboration with an established research group.

In accordance with Dr Smith's will, candidature is limited to candidates who have obtained their PhD degree, or equivalent, within the last three years at any university but normally excluding Cambridge and Harvard.

The stipend will be on the University's Postdoctoral Research Associate scale, currently between £28,132 to £36,661 per annum, with a research allowance of £15,000 (£13,000 in Pure Mathematics) in the first year and (currently) £11,000 p.a. thereafter.

Further details are available at <http://www.herschelsmith.cam.ac.uk/fellowships/> or from the Secretary to the Fund Managers, Tel. +44 (0)1223 332283, e-mail duncan.mccallum@admin.cam.ac.uk.

Please quote reference AK02310 on your application and in any correspondence about this vacancy.

Closing date: 10 January 2014

The University values diversity and is committed to equality of opportunity.

The University has a responsibility to ensure that all employees are eligible to live and work in the UK.



TWO FACULTY POSITIONS in SYNTHETIC ORGANIC CHEMISTRY and CELLULAR/SYSTEMS PHYSIOLOGY

The Department of Chemistry, Chemical Biology, and Biomedical Engineering at the **Stevens Institute of Technology** invites applications for **two tenure-track** positions at the level of **Assistant or Associate Professor** in the areas of (1) synthetic organic chemistry and (2) cellular or systems physiology. The successful applicants will hold a PhD with postdoctoral experience and a good publication record. It is our expectation that the successful candidates will develop dynamic, externally-funded research programs and will teach undergraduate and graduate courses. We are particularly interested in attracting candidates with research interests that complement our existing institutional strengths in development and delivery of novel therapeutic agents and tissue engineering and mechanics. The Department's undergraduate and graduate programs in Chemistry and Chemical Biology and in Biomedical Engineering enjoy interactions with New Jersey and New York City metropolitan area academic, healthcare, and pharma/biotech institutions and host over 300 highly motivated undergraduate and graduate students. The Stevens Institute of Technology has a 140-year history of advancing engineering, science, and technology.

For full consideration, please submit three letters of reference as well as a single electronic file containing your Curriculum vitae, research plan, and teaching statement to **Dr. Philip L. Leopold** [e-mail: pleopold@stevens.edu] by **January 31, 2014**.

Women and members of under-represented groups are encouraged to apply. The Stevens Institute of Technology is an Equal Opportunity/Affirmative Action Employer.



HUMAN FRONTIER SCIENCE PROGRAM

CALL FOR LETTERS OF INTENT
FOR RESEARCH GRANTS:
AWARD YEAR 2015

HFSP supports **international** preferably **intercontinental** collaborations in **basic** life science research. Applications are invited for grants to support **innovative** approaches to understanding **complex mechanisms of living organisms**. Applicants are expected to develop **novel** lines of research distinct from their ongoing research. Preliminary results are not required.

Program Grants are for independent scientists at all stages of their careers while **Young Investigators' Grants** are for teams of scientists who are all within 5 years of establishing an independent laboratory and within 10 years of obtaining their PhDs. Both provide 3 years support for 2 – 4 member teams, with not more than one member from any one country, unless critical for the innovative nature of the project. Awards are dependent upon team size and successful teams will receive up to \$450,000 per year. The principal applicant must be located in one of the HFSP member countries but co-investigators may be located in any country.

For further information see our web site (www.hfsp.org). Teams must register via the web site by March 20th 2014 so as to submit a letter of intent online by the March 27th 2014 deadline.

Specific enquiries: grant@hfsp.org

Research, a promise of future

Inserm is the only French public research institute to focus entirely on human health. Its researchers are committed to studying all diseases, whether common or rare. Through its diversity of approaches, Inserm provides a unique environment for researchers. 13 500 researchers, engineers and technicians work in the 289 Inserm laboratories housed in hospitals, universities and research campuses, all over France.



**Inserm is recruiting
114 tenure positions
are offered to researchers (m/f)
dedicated to biomedical research**

Candidates to Research Associates and Research Directors positions must have a PhD (or equivalent degree). There is no nationality restriction.

Application modalities: visit our website : www.eva2.inserm.fr

Application deadline:

- Research Associates: **January 17th, 2014 - 4.00.pm (GMT+1)**
- Research Directors: **February 28th, 2014 - 4.00.pm (GMT+1)**

Instituts
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AIDS Conference
Melbourne, Australia

July 20-25, 2014

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There's only one

DR. SHIRLEY MALCOM



To Dr. Shirley Malcom, born and raised in the segregated South more than 65 years ago, a career based on her studies in science seemed even less likely than the launch of the Soviet's Sputnik. But with Sputnik's success, the Space Race officially started and, in an instant, brought a laser-like focus to science education and ways to deliver a proper response. Not long after, Dr. Malcom entered the picture.

Although black schools at the time received fewer dollars per student and did not have sufficient resources to maintain their labs at a level equivalent to the white schools, Dr. Malcom found her way to the University of Washington where she succeeded in obtaining a B.S. in spite of the difficulties of being an African American woman in the field of science. From there she went on to earn a Ph.D. in ecology from Penn State and held a faculty position at the University of North Carolina, Wilmington.

Dr. Malcom has served at the AAAS in multiple capacities, and is presently Head of the Directorate for Education and Human Resources Programs. Nominated by President Clinton to the National Science Board, she also held a position on his Committee of Advisors on Science and Technology. She is currently a member of the Caltech Board of Trustees, a Regent of Morgan State University, and co-chair of the Gender Advisory Board of the UN Commission on Science and Technology for Development. She has held numerous other positions of distinction and is the principal author of *The Double Bind: The Price of Being a Minority Woman in Science*.

Of her active career in science, Dr. Malcom says, "I guess I have become a poster child for taking one's science background and using that in many other ways: we ask questions; we try to understand what we find; we consider what evidence we would need to confirm or refute hypotheses. And that happens in whatever setting one finds oneself."

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蘇州大學

SOOCHOW UNIVERSITY

Molecular Imaging and Nanomedicine Research

The School of Radiation Medicine and Protection (SRMP) and School of Radiological and Interdisciplinary Sciences (RAD-X) of Soochow University Medical College in Suzhou, China are seeking outstanding candidates for postdocs and faculty positions at associate and full professor levels in the **Division of Molecular Imaging and Nanomedicine**, which is devoted to promote scientific and technological innovations in advanced imaging techniques for the theranostics of major diseases. Candidates are expected to have interdisciplinary research experience with strong background in Tumor Biology, Cell Biology, Immunology, Nuclear Medicine, Optics, and Imaging Science.

Qualifications for postdocs include a Ph.D. and research experience in the related fields. Associate Professor Candidates should have a Ph.D. and >3-year postdoctoral experience. Candidates for full professor positions should have excellent publication and external funding records. All candidates must have a good command of written & spoken English.

Successful appointees will be offered with an excellent package including sufficient lab space, start-up funding, relocation aid, competitive salary commensurate with experience, and other employee benefits.

Please submit (i) a cover letter summarizing current research projects and future plans, (ii) a curriculum vitae, and (iii) names and contact info of 3 professional referees to: **SRMP and RAD-X Search Committee, Soochow University, Dushu Lake Campus, 199 Ren-Ai Road, Suzhou 215123, China; Email: bxzhu@suda.edu.cn**

ANNOUNCEMENTS

Single Protein Dynamics in *Cellulo* 2014: Spatio-Temporal Structural and Quantitative Analyses

April 21 – 25, 2014

Okinawa Institute of Science and Technology
Graduate University

Okinawa, Japan

* No registration fee.

* We will handle the logistics and cover the cost of twin-share accommodation at Seaside House and meals for all workshop participants.

* We will also help with arranging visas when necessary.

* We envision that we will be able to offer full financial support for most participants (twin-share accommodation, meals and return airfare).

* We request that all applicants indicate on the application form whether they wish to apply for full travel support.

** For more information, please visit website **

<https://groups.oist.jp/spdc>



Federal Ministry
of Education
and Research



“Bernstein Award” 2014 Young Scientists Research Award in Computational Neuroscience

The German Federal Ministry of Education and Research (BMBF) has established the “National Network for Computational Neuroscience” with six high-performing “Bernstein Centers for Computational Neuroscience” as the major structural elements.

The “Bernstein Award” is equipped with up to 1.25 Mio Euros in the form of a grant over a period of five years. It will be awarded to a highly qualified young researcher, considering the candidates' verifiable research profile in the field of Computational Neuroscience and the scientific concept for a future young research group. Young researchers can apply for their own position and group. The group funded by the “Bernstein Award” will become an integral part of the National Network for Computational Neuroscience. Future announcements of the “Bernstein Award” are in the scope of the Ministry's planning.

The grant is provided for a scientific project of a young research group headed by a postdoc regardless of nationality. The project will be conducted at a German university or research institution – within or outside the Bernstein Centers. It is a prerequisite for funding that the university or research institution concerned employs the young researcher during the funding period and supports him/her with the basic equipment in terms of laboratory space and other infrastructure. A statement made to that effect by the receiving institution must be included with the project outline to be submitted.

Deadline for applications is April 15th, 2014

For more detailed information about the “Bernstein Award” including application conditions please visit

<http://www.nncn.de>

or

http://www.gesundheitsforschung-bmbf.de/_media/Bernstein_Award_2014_Call_for_proposals

POSITIONS OPEN

CHAIRPERSON

Department of Microbiology and
Molecular Genetics
Michigan State University

Michigan State University (MSU) invites applications and nominations for the position of Chairperson, Department of Microbiology and Molecular Genetics. This large, vibrant, and diverse department has over 40 faculty members who conduct research in all domains of life and has outstanding graduate and undergraduate programs. Focus areas include genomics and molecular genetics; immunology and virology; microbial evolution, ecology and physiology; and molecular pathogenesis. The department serves the needs and interests of the College of Veterinary Medicine, College of Human Medicine, College of Natural Science, College of Osteopathic Medicine, and Michigan AgBioResearch. The department is located in a new, state-of-the-art building that also houses the Departments of Physiology and Physics, and is connected to buildings housing the Department of Biochemistry and Molecular Biology and Department of Chemistry.

The Chairperson will provide leadership for the department and interdisciplinary activities on campus. We seek an individual with an internationally recognized research program that has been consistently externally funded who also has demonstrated administrative and interpersonal skills. The person should have a strong commitment to graduate, undergraduate, and professional education, and an appreciation for the diverse areas of expertise in the department. Continuation of an active research program in any relevant area is expected and will be supported.

Application materials must include a statement of interest highlighting specific strengths related to this position including previous administrative experience and accomplishments, research interests and plans, funding history, and a statement about commitment to diversity. Include also curriculum vitae and names of three references (who will not be contacted without your permission). All materials should be assembled into one PDF (less than 2 MB). The PDF should be uploaded to **website: <https://jobs.msu.edu>** (posting # 8796).

Questions regarding this position may be sent to the Chair of the Search Committee, **Tom Sharkey**, at e-mail: mmgchairsearch@cns.msu.edu. Review of application materials will begin on February 1, 2014 and continue until a suitable candidate is identified. The search committee is committed to respecting and maintaining confidentiality until a list of candidates for interviewing is determined.

MSU is an Affirmative Action/Equal Opportunity Employer. MSU is committed to achieving excellence through cultural diversity. The University actively encourages applications and/or nominations of women, persons of color, veterans, and persons with disabilities.

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